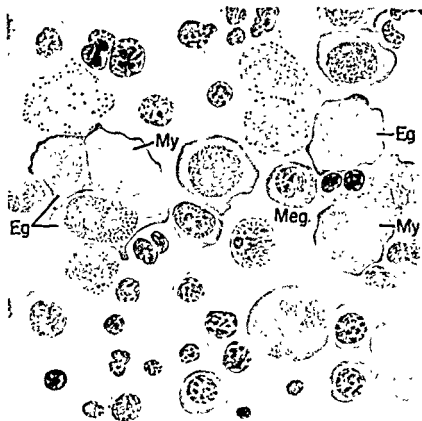


LEUKEMIA



Y KAWAKITA

FRONTISPIECE

D₁ Guglielmo's syndrome (erythremic myelosis), an acute form of the myeloproliferative syndrome. Smear of bone marrow (Wright-Giemsa stain) showing large numbers of primitive nucleated red cells (erythrogones *Eg*) with a megaloblastoid (*Meg*) type of development. There is also an increase in primitive and young granulocytes *My* myeloblast. Magnification, $\times 715$

LEUKEMIA

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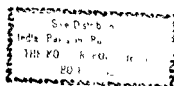
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Preface

LEUKEMIA, like cancer and poliomyelitis, has been classed as one of the "dread diseases." Without doubt, it represents the most important single problem in hematology. In the United States alone it kills at least 10,000 annually, many of them bright, active children or intelligent men and women in their prime of life. Most statistics indicate that the disease is on the increase, particularly in the last three decades of life. Whether or not this is actually true or due simply to more case studies and better recognition, there can be no question regarding the seriousness of the problem and the necessity to cope with it by all available means.

There have been many thousands of articles written about leukemia but the paucity of books on the subject is amazing. Forkner's text of 1938 was encyclopedic in its scope and for many years remained almost the only central source. The enormous resurgence of interest in the disease, brought about in large measure by the possibility of achieving at least temporarily beneficial results with various chemicals, has led to a quest for more precise knowledge of the disease—its character, the nature of the leukemic cell, the pathophysiology of such features as the anemia, hyperuricemia, the hemorrhagic state, etc. Etiologic factors, previously unknown, have come to the surface, and today there is great talk of the viruses and much statistical evidence for the leukemogenic effects of ionizing radiation. The empirical nature of most of our therapy, even that with the newer antimetabolic and cytotoxic agents, and its eventually unsatisfactory characteristics, have naturally led to an increasing inquiry into the more fundamental aspects of cellular growth and proliferation.

What is leukemia? Is it a reactive disturbance, or is it neoplastic? Does it represent a cellular reaction to an infectious or other agent, or does a harmful mutation take place, leading to an abnormal type of unusually rapid leukocyte proliferation? The leukemic cell seems to have some rather characteristic features as we examine it, but when one tries to analyze it feature by feature, chemical by chemical, the apparent differences between normal and leukemic cells become less and less pronounced. Perhaps this is why, in treating leukemia, we are always limited by what the chemical or other agent does to the *normal* cells, the action upon both leukemic and normal cells is so much alike.

This work on leukemia is limited almost entirely to a consideration of *human* leukemia. Not that mouse leukemia and fowl leukemia are not important, they are of utmost importance, particularly from the investigational aspect. We present in this monograph a rather personal account, not only of our own interests in this field but of what we think the practitioner (internist, pediatrician, pathologist and clinical pathologist) may be interested in. The work is by no means encyclopedic nor is it a textbook, although sometimes, as in the clinical descriptions, it must partake of some of the features of the latter. There is probably more emphasis on certain aspects than on others, again an indication of our special fields of interest—etiologic agents, the myeloproliferative syndromes, therapy. Nevertheless, we believe that there is presented in these pages a fairly comprehensive picture of the present state of our knowledge (some might say

"ignorance") of leukemia. We realize full well that this is but an interim report and that perhaps in a short time, whether it be a year or a decade, a revolution in understanding and control of the disease may well take place. Actually, the fact that one has a difficult time in defining leukemia may in itself be somewhat hopeful. Since there is no complete certainty that the condition is malignant, nor even what "malignancy" is, it is altogether possible that leukemia may eventually turn out to be a deficiency state or an immunologic reaction or a response to an infectious agent. Again, what we learn from leukemia, with its readily available blood and tissue cells, should certainly be of considerable value in the understanding of neoplastic disease in general.

This work could never have been completed without the help of many individuals. From our patients we have learned a great deal, particularly in courage and forbearance. From our colleagues, who have come to work with us from many lands and many parts of this country, we have gleaned much valuable information, and the give-and-take of our daily discussions has been of utmost value. We may single out a few who have worked with us on specific problems in this field: Drs. Mario Baldini, Boston; Luis Bergna, Buenos Aires; Marvin Bloom, Buffalo; Edmund W. Campbell, Boston; Jyoti Chatterjea, Calcutta; William H. Crosby, Washington, D. C.; Solomon Estren, New York; Henry Goldenberg, Toronto; Norma Granville, Hartford; Zacharias Komninos, New York; William McFarland, U S Navy, Bethesda, Maryland; Carlos Mesa Arrau, Santiago, Chile; Enrique Perez Santiago, Santurce, Puerto Rico; Anthony Pisciotta, Milwaukee; Jack Rheingold, Washington, D. C.; Martin Rosenthal, New York; Fernando Rubio, Jr., Boston; Richard H. Saunders, Rochester; Laurence I. Schwartz, New York; Jay Silverberg, Pittsburgh; Karl Singer, deceased; Mario Stefanini, Boston; Asuman Unugur, Istanbul; Louis Weisfuse, Long Island, New York; Leda Zannos, Athens, Greece.

In addition, we wish to acknowledge with thanks the help of the following individuals, all of New Zealand: Drs. G. C. T. Burns, A. F. Burry, A. J. Campbell, A. M. Goldstein, R. F. Hough, J. B. Jameson, G. L. Rolleston, D. T. Stewart, and Messrs. S. E. Brooks and K. A. Donaldson.

Our secretarial staff headed by Miss Joyce Rock and including Mrs. Arlene Morris, Mrs. Mildred Seagraves and Miss Zelda Cushner, has somehow triumphed over a mountainous collection of drafts, copies, bibliographies, illustrations, made all the more complex by the half-world separating the two authors. Among our many technicians over the years, we must particularly note Mrs. Irma B. Mednicoff and Mrs. Louise D'esy Choinski.

Special thanks are due to Dr. H. Edward MacMahon, Professor of Pathology, Tufts University School of Medicine, and his staff for their cooperation in providing most of the photomicrographs of sections in the text (except those otherwise cited), Dr. Alice Ettinger, Radiologist, New England Center Hospital, for her continued interest in our work, and for supplying most of the x-ray illustrations used in the text, Dr. W. J. Mitus of our laboratory, for his aid in the histopathologic and histochemical sections, Professor Y. Kawakita of Kumamoto City, Japan, for his meticulous illustrations done while he was with us in Boston, Dr. Joseph Beard of Durham, North Carolina, who reviewed the section on viruses and with whom we had several profitable discussions on this important subject. Dr.

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We acknowledge with appreciation the help of our wives, Mrs. Ruddy Dameshek and Mrs. Joan P. Gunz, whose patience was undoubtedly strained at times while their husbands toiled over an ever-demanding manuscript. To our long-time secretary, Mrs. Edith M. Florentine, we extend thanks for her constant cooperation, particularly in unearthing pertinent clinical data.

Finally, we cannot fail to acknowledge the generous support throughout the years of the American Cancer Society, Inc., the American Cancer Society (Massachusetts Division), the National Cancer Institute of the United States Public Health Service, the Medical Research Branch (Division of Biology and Medicine) of the United States Atomic Energy Commission, the Damon Runyon Memorial Fund for Cancer Research, Inc., and of generous private donors. Among these may be listed Mr. E. Calvin Fowler, of Chattanooga, Tennessee (deceased May 29, 1958), Mr. E. Stanley Wright of Worcester, Massachusetts, the Greenbaum Family of Boston (in honor of Mrs. Sarah Greenbaum, deceased), and the Rho Phi Phi Ladies Auxiliary, of Boston. We are indebted to the Schering Corporation and to its Vice-President and Medical Director, Dr. Edward Henderson, for their generous contributions of large amounts of prednisone (Meticorten) and their financial aid in defraying the cost of the colored plates.

*William Dameshek
Frederick Gunz*

I. Leukemia in the Past

LEUKEMIA, since its recognition as a distinctive disease, has had a history of little more than 100 years, and it is therefore a comparative newcomer among the major known scourges of humanity. It was first described almost simultaneously by two brilliant young men who, after applying their great gifts to a meticulous exploration of its features in the living and the dead, engaged at once in an almost venomous wrangle over the honor of having been the first to identify this fatal disease. Progress in the knowledge of leukemia has been fitful since these beginnings. It should be realized that at the time of its discovery very little was known about the composition, the origins and the functions of normal blood, nor were there any good methods available for investigating them. Each step forward had therefore to be preceded by an exploration of the normal. Where this lagged, speculation usurped the place of research and theory that of fact. Much of the literature of the first hundred years echoes with the clash of controversy which, lacking a basis of substantial facts, could only be dialectic and unproductive. It is regrettable but true that even today we do not possess the answers to many of the fundamental questions about the mechanism of normal hematopoiesis or the regulation of the blood elements. This fact largely explains our continuing ignorance of the causes of leukemia and of the means of subjugating it.

It seems likely that the first accurate description of a case of leukemia was given in 1827 by Velpeau.¹⁷ His patient, a 63 year old florist and seller of lemonade, "who had abandoned himself to the abuse of spirituous liquor and of women without, however, becoming syphilitic," fell ill in 1825 with a pronounced swelling of the abdomen, fever and weakness, and symptoms caused by urinary stones. He died soon after admission to hospital and at autopsy was found to have an enormous liver and spleen, the latter weighing ten pounds. The blood was thick, "like gruel, . . . resembling in consistency and color the yeast of red wine . . . One might have asked if it were not rather laudable pus, mixed with blackish coloring matter, than blood." It was in fact the peculiar character of the blood, as seen post-mortem, which first attracted the attention of all the early observers of leukemia. Thus Barth,² in 1839, was so interested in the autopsy findings in one of his patients that he submitted the blood to microscopic examination. This was carried out by Donné, who reported that more than half of the blood consisted of "mucous globules" which could not be distinguished from pus corpuscles. It appears that Donné¹⁹ was the first to examine the blood of another leukemic patient during life; it was so full of colorless corpuscles that at first he thought it was pus.

In spite of these and other early observations, leukemia was not recognized as a definite entity until its description in 1845 by Bennett⁴ in Scotland and by Virchow²⁰ in Germany. The independent publication, within one month of each other, of two cases of the same new disease, was less remarkable than the fact that each observation came from the pen of a man who was to become a leader in his own field, Bennett in physiology and Virchow in pathology. In each of the two cases it was the post-mortem appearance of the blood which first gave the

and diagnosis of diseases of the blood should be based on a consideration of the patient as a whole, not on examination of the blood alone.

With these features of hematology in mind, a brief discussion of the structures and functions of the components of the hematopoietic system may clarify the part these organs play in maintaining a normal blood picture, and render more easily understandable the effect on the blood of pathologic changes that they may undergo.

RETICULO-ENDOTHELIAL SYSTEM

The term *reticulo-endothelial system* was proposed by Aschoff to designate collectively a group of specialized cells widely scattered throughout the body, in many organs and tissues intermingled with other types of cells. Reticulo-endothelial cells are derived from embryonic connective tissue, the mesenchyme, which is the source of the earliest blood cells in the embryo. At different periods in fetal development and in different locations the mesenchyme gives rise to all forms of blood cells. Remnants of the mesenchymal cells persist throughout life and maintain their potential ability to form blood cells. They differ widely in their structural characteristics, and are variously known as clasmatocytes, hemohistioblasts, or macrophages. They have in common the ability to phagocytose foreign particles. This power of phagocytosis accounts for their specific supravital staining properties, particles from a weak solution of a dye are phagocytosed and assembled within the cell into such large groups that they become visible under the microscope. Stained with specific dyes, cells of the reticulo-endothelial system can be detected among ordinary tissue cells which are not phagocytic. Because of their common properties and similarity of function, these cells are grouped together and are considered as a separate organ even though they are widely dispersed throughout the body.

The *reticulo-endothelial cells* may be separated into two groups, fixed cells and wandering cells

Fixed Cells

Fixed cells are found primarily in the following sites: (1) in common connective tissue and serous membranes, where the cells may be round, spindle-shaped, or large, irregular, branching forms, ordinarily they lie among the fibroblasts but they may become free to wander through the tissues; (2) in the reticula of the spleen, lymph nodes, and bone marrow, where they are of large size and have branching processes by which they

are attached to one another and to the reticular stroma of the organ; (3) lining the blood sinuses of the spleen and bone marrow, where they are flat and endothelial-like. Similar to these cells are the large, flattened, stellate cells (Kupffer's cells) which occur in the blood sinuses of the liver. Cells of both types have many branching processes which project into the lumen of the blood capillaries and are so insecurely anchored that the cells may break loose and become free in the blood channels.

Wandering Cells

Wandering or free reticulo-endothelial cells may be found in either the tissues or the blood stream. The fixed histiocytes in the connective tissue may become actively motile—particularly as a result of an inflammatory stimulus—and may then move about through the tissues. Such cells are observed most commonly in the spleen, lymph nodes, bone marrow, and omentum. The large endothelial-like cells which are found in the walls of the blood sinuses occasionally break loose and become free in the circulation, but because of their size they are usually filtered out of the circulation as they pass through the lungs. Monocytes that occur in the blood stream are of reticulo-endothelial origin and belong to this system.

The role of the reticulo-endothelial system in formation of monocytes of the blood stream and in removal and destruction of degenerated cells, especially degenerated erythrocytes, makes it of special interest to the hematologist. The spleen, which is particularly rich in reticulo-endothelial tissue, is the organ chiefly involved in these functions. Not all erythrocytes are phagocytosed by the reticulo-endothelial cells; many of them probably undergo disintegration or fragmentation within the blood stream as a result of stresses and strains to which they are subjected. The fragments are taken out of the circulation by the reticulo-endothelial cells. Since these cells can also remove colloidal substances from suspension, they take from the blood stream the hemoglobin liberated by disintegration of the erythrocytes. Reticulo-endothelial cells are able to break down hemoglobin to the stage of bilirubin; thus the blood leaving those organs which have a large amount of reticulo-endothelial tissue has a significantly higher bilirubin content than does arterial blood. Although the hepatic cells are responsible for elimination of bilirubin, they are not concerned with its formation.

In addition to destruction and removal of erythrocytes and formation of monocytes, the reticulo-endothelial cells remove all forms of particulate matter from the blood stream, and in this capacity they form a vital part of the defense against bacteria. They contribute to the repair process

which follows tissue injury and inflammation. In the lungs they remove foreign particles that have entered the body through the respiratory passages. In the liver the Kupffer cells phagocytose thorium dioxide to such an

DECREASE IN RED BONE MARROW WITH ADVANCING AGE

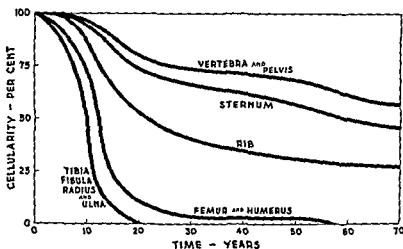


FIG. 1. The approximate rate of decrease in the cellularity of the bone marrow with advancing age (Redrawn from data obtained by Whitby and Britton, Disorders of the Blood, J & A Churchill)

extent that this organ can subsequently be visualized roentgenographically. As the cells have the power to store material they cannot digest, they are the depository for ingested iron which is not used immediately in the formation of hemoglobin. In certain metabolic disturbances such as Gaucher's disease and Niemann-Pick disease they store large amounts of lipoid substances. Antibacterial substances and antibodies in the blood serum are probably chiefly derived from the reticulo-endothelial cells.

BONE MARROW

The medullary cavities of bones in the adult contain two types of marrow tissue. the hematopoietic portion, or the red, cellular marrow in which blood cells are formed, and the fatty or yellow marrow. In the fetus at the time of birth the entire marrow cavity of all bones is filled with red, cellular marrow, with advancing age this is replaced by yellow marrow, which appears first in the centers of the long bones and progresses distally and proximally from these points. Replacement occurs first in the more distal bones. The fatty marrow gradually increases in extent until at about the age of

twenty years all the marrow of the long bones is of this type except that in the proximal ends of the femurs and humeri. Red marrow persists in the flat bones, hence hematopoiesis is most active in the ribs, sternum, vertebral bodies, pelvis, and skull. Figure 1 shows the progress of replacement of red by yellow marrow as it occurs in the bones with advancing age.

The fatty or yellow marrow consists of fat cells, blood vessels, and a minimal amount of reticular framework. Although blood cells are not formed in this portion of the marrow, there is ample room for expansion of the red marrow into the shafts of the long bones, and under proper stimulation fatty marrow may be replaced by hematopoietic marrow.

The red marrow is the portion in which the various types of blood cells are produced (Fig. 2); it is especially rich in reticulo-endothelial cells. It is estimated that the active marrow in the adult comprises from 3.5 to 6 per cent of the body weight or from 1500 to 3500 grams which is approximately the weight of the liver. The volume of the marrow ranges from about 70 c.c. at birth to 4000 c.c. in the adult but only about half of this is active. Since all of the marrow is already active in hematopoiesis in the fetus and newborn infant, an additional stimulus for red cell formation results in extramedullary blood formation, whereas in the adult there is an extension of the hematopoietic marrow into the yellow fatty portion. The bone marrow is commonly considered as a single organ even though it is scattered through the cavities of many bones. The red marrow presents a delicate reticulum of connective tissue supporting numerous vascular channels and cellular elements. Lymphoid tissue is scanty but most observers look upon lymph follicles in the bone marrow as normal. The circulation is of the closed type, and the vascular bed is a meshwork of small sinusoids lined with flat endothelial-like cells similar

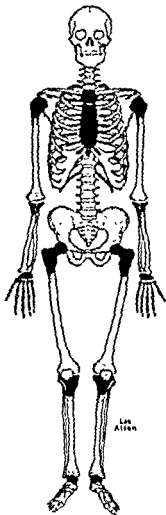


FIG. 2 Hematopoietic bone marrow in the adult. The shaded and darkened portions of the skeletal system represent the areas in which red or hematopoietic marrow persists in the normal adult.

to those lining the sinuses of the spleen. These lining cells are in intimate contact with the stellate cells of the supporting reticulum, which also belong to the reticulo-endothelial system. Under normal conditions many of the sinusoids are completely collapsed and contain no blood, while others are dilated but, owing to constriction of the efferent portion, are so shut off from the circulation that a low oxygen tension results. Sabin believes that the red blood cells are formed in these sinusoids, the flat endothelial-like cells dividing and subdividing into immature erythrocytes under the stimulus of the anoxemia. Supported by the delicate reticulum are other types of marrow cells, including the precursors of the granulocytes and megakaryocytes as well as other large, branched cells of the reticulo-endothelial system. Whereas the erythrocytes are believed by many investigators to be formed intravascularly, the granulocytic or myelocytic series of cells are produced outside the vascular channels. Downey and others believe that the erythrocytes are likewise developed extravascularly and originate from the general reticulum rather than from that lining the sinusoids. In the reticulum of the red marrow are large cells with branching processes which fuse together to form a loose network. These are the nearest approach to the primitive mesenchyme cell to be found in the adult, and from them the granulocytes and possibly the erythrocytes originate. Megakaryocytes also arise from the reticular cells, although they may likewise be formed from the cells in the sinusoids.

SPLEEN

The spleen is an important part of the hematopoietic system and as such it serves three well recognized functions: (1) It destroys and removes blood cells and platelets from the circulation; (2) it serves as a reservoir for storage of a reserve supply of erythrocytes; and (3) lymphocytes are formed within its malpighian corpuscles. In addition to these hematologic functions it has a role in the protective mechanism of the body against infections; it is the site of formation of antibodies, it is active in phagocytosis and removal of bacteria and other particulate matter from the blood stream, and it has other less well defined functions.

The spleen measures about 12 cm. in length, 7 cm. in width, and 3 cm. in thickness. With an average weight of from 150 to 200 Gm., the spleen during life is considerably larger and heavier than is the organ which is examined at necropsy. It is reddish purple in color and pliable in consistency. It is enclosed in a connective tissue capsule from which trabeculae extend into its

substance, tending to divide the organ into imperfect and irregular lobules. The elastic fibers and bundles of smooth muscle in both the capsule and the trabeculae render the organ capable of contraction. From the trabeculae arises a fine reticular framework which supports the splenic pulp and the nodules of lymphoid tissue—the malpighian corpuscles or splenic nodules. Enmeshed within the pulp are many types of cells. Erythrocytes predominate, but there are also granulocytes, lymphocytes, and plasma cells. In addition to these blood cells there are large mononuclear cells which are motile and have phagocytic properties—the macrophages. The blood is delivered to the splenic pulp by fine arterial vessels, and as it percolates slowly through this spongelike structure, it is in intimate contact with the reticulo-endothelial cells, which are numerous in the pulp and the reticular framework of the spleen. The blood is collected in venous sinusoids whose walls are composed of spindle-shaped cells separated by slitlike spaces allowing the blood to have free communication between the pulp and the sinusoids.

Scattered throughout the spleen are islands of lymphoid tissue, the malpighian corpuscles, which have a pale central area known as the germinal center. The malpighian corpuscles are similar in structure and function to lymph nodes elsewhere in the body.

Function of the Spleen in Relation to Hematology

Macrophages are large ameboid cells of the splenic pulp frequently containing fragments of, or even whole, erythrocytes. In conditions in which there is excessive destruction of red blood cells, the phagocytic macrophage cells may engulf unusually large numbers of fragmented erythrocytes, with the result that the spleen ultimately becomes impregnated with the iron and iron-containing pigment, hemosiderin, derived from the hemoglobin of the destroyed cells. It is probable that the phagocytic process in the spleen removes only fragmented or damaged erythrocytes and not healthy cells. The spleen is not the only organ concerned with the removal of erythrocytes as all parts of the reticulo-endothelial system apparently partake in this function. The process of destruction and removal of erythrocytes continues after splenectomy but in the normal individual the spleen appears to be the primary site of erythrocytic destruction.

That the spleen is active in the removal of other circulating elements from the blood stream is suggested by the effects of splenectomy on the leukocyte count and platelet count in splenic neutropenia and thrombopenic purpura. There is some question as to the exact role the spleen normally plays in the

removal of these elements but it seems probable that their removal is due in part to this organ.

The increase in the hemoglobin concentration and erythrocyte level of the blood which occurs under certain conditions is too rapid to be explained on the basis of production of new hemoglobin and red blood cells. The concomitant reduction in the size of the spleen indicates that its reserve supply of erythrocytes is released into the circulation to meet additional demands, for example, during strenuous exercise. A similar reaction occurs with hemorrhage, reduced atmospheric pressure, and asphyxia but the splenic function as a reservoir is apparently not as important as it was once believed since a similar increase in erythrocytes occurs in splenectomized individuals. The blood flow through the splenic pulp is normally so sluggish that the stored cells are not delivered to the blood stream, but by contraction of the smooth muscle bundles in the framework of the spleen its size is reduced, thereby forcing the cells into the general circulation. This process can be reproduced by injections of adrenalin. During surgical removal of the spleen the entrapped erythrocytes may be forced into the circulation by manual pressure on the organ before its vessels are ligated.

Lymphocytes are produced in the malpighian corpuscles, as they are in other lymphoid tissue in the body. Circulating monocytes are formed from the reticulo-endothelial tissues of the body, and since the spleen is particularly rich in this tissue, it is one of the principal sites of their development. Except for these cells the spleen is not a site of hematopoiesis in the normal adult. Under the influence of certain stimuli the spleen may resume its fetal function and areas of extramedullary hematopoiesis make their appearance.

Iron is stored in the cells of the reticulo-endothelial tissues throughout the body, including those of the spleen, where because of the numerous reticulo-endothelial cells the amount of iron stored is correspondingly large. It has been demonstrated that splenectomy greatly reduces the reserve supply of iron in the body.

During fetal life the spleen is actively engaged in formation of erythrocytes, but this function becomes dormant when production of these cells is taken over by the bone marrow. However, the spleen retains its potential ability to form erythrocytes, as well as cells of the myelocytic series, and under certain pathologic conditions its reticulo-endothelial cells may revert to this fetal function with resultant extramedullary hematopoiesis. This process takes place in erythroblastoses, and the extremely large numbers of myelocytic cells found in the spleen in myelogenous leukemia may be the result of metaplasia rather than of infiltration.

Effects of Splenectomy

Although the spleen has been considered an important hematopoietic system and it has been shown in some body it is not an essential organ as has been amply demonstrated by the effects of splenectomy. Many of its functions are taken over by other portions of the reticulo-endothelial system after its removal so that there is no appreciable decrease in antibody production nor apparent increase in the patient's susceptibility to infection.

It has been shown in animals that a transient anemia develops after the spleen is removed and this has been reported to occur in humans after the operation but it is not consistently encountered. Howell-Jolly bodies in the erythrocytes is a common finding in the blood of a splenectomized individual and suggests an abnormality in nuclear extrusion, indicating that the spleen is in some way concerned with this process. Target cells are present in increased numbers after the spleen is removed. These cells are thinner than normal and their increase in the blood stream may account for the increased resistance of the erythrocytes to hypotonic saline following splenectomy. There is usually a lowered urobilinogen output in the feces indicating a diminution in hemoglobin destruction.

There is an immediate postoperative increase in blood platelets, the number frequently rising to over 1,000,000 per cubic millimeter and the volumetric determination to 3 per cent or higher. After reaching a peak the number of platelets subsides to the normal level, although it may remain above normal for several weeks or months.

There is also a postoperative leukocytosis, the white cell count being commonly 20,000 to 40,000 per cubic millimeter with an increased number of young forms. The high leukocyte count is due almost entirely to an increased number of neutrophils. The leukocytosis disappears slowly and may persist for many weeks. Eosinophilia occurs occasionally, and a lymphocytosis has been observed.

It has been noted that persons whose spleens have been removed tire more easily on exertion than do normal persons.

The role that the spleen plays in the regulatory mechanism of hematopoiesis is not well understood. Attempts have been made to prove the existence of a hormone or hormones in the spleen which regulate the activity of the bone marrow and the production of blood platelets, granulocytes, and erythrocytes. Various extracts have been produced which seem to exert some influence on the platelet or leukocyte content of the blood but these studies need confirmation before the existence of a splenic hormone can be accepted. The

term "hypersplenism" has been returned to use in recent years in certain conditions in which one or more of the physiologic functions of the spleen are overactive but whether this overactivity is hormonal in nature is not known. The overactivity is manifest by a reduction in any one or all of the cellular blood elements: the erythrocytes, leukocytes, or platelets. It has not been determined whether this reduction results from increased destruction of the cells or to decreased production. Removal of the spleen in certain cases corrects the deficiency but does not answer the question as to how it is accomplished.

LYMPH NODES

Lymphoid tissue is found in all parts of the body as (1) diffuse masses, (2) simple nodules, and (3) large and complex lymph nodes. It is composed of supporting reticulum containing lymphoid cells within the meshes of its framework. The strands of the reticulum vary in size. In areas in which the lymphoid cells are particularly numerous the reticular network is almost hidden. The simple lymph nodule, such as occurs in the walls of the intestinal tract, is irregularly spherical or elliptic in shape. It is composed of a mass of lymphoid tissue with a dense peripheral zone enclosing a central area of looser and lighter texture known as the germinal center. Within it are lymphoid cells in all stages of development. Since the lymph nodule lacks a distinct capsule, its outer border is imperfectly defined by the surrounding connective tissue.

Lymph nodes are interspersed along the lymphatic vessels, usually embedded in fatty tissue. They are more highly differentiated organs than the lymph nodules. They vary in size from minute bodies to oval- or bean-shaped organs measuring 2 cm. or more in length. They are enclosed in distinct fibrous capsules which consist in part of elastic and smooth muscle fibers. Trabeculae from the capsule penetrate the outer portion or cortex of the gland, dividing it into pyramidal compartments. In the central or medullary part of the gland the trabeculae are less regularly distributed, but tend to divide this portion into cylindrical compartments. These poorly defined cylindrical areas—the medullary cords—contain masses of lymphoid cells. They are continuous with one another and communicate with the cortical portions of the lymph node. Interspersed between the lymphoid tissue and the trabecular framework are the intercommunicating lymph sinuses, lined with an imperfect layer of flattened endothelial cells. Within the lymph sinuses is a reticulum which is continuous with the adjacent reticular frame-

work of the gland. The blood vessels entering the hilus of the gland subdivide to form a rich capillary network throughout the cortical nodules and the medullary cords. Germinal centers occur only in the cortical nodules.

The lymphocytes of the blood stream are developed in the lymphoid tissue throughout the body, not only in the germinal centers of the lymph glands but also in the lymphatic tissue that is so widely distributed in the gastrointestinal tract, thymus, tonsils, and elsewhere. The minute lymphatic nodules which are present in most organs of the body also take part in the production of these cells. Large numbers of reticulo-endothelial cells are present in the reticulum of all lymphoid tissue, and the precursors of the lymphoblasts are derived from the indifferenciated primitive reticular cells of the stroma. Lymphocytes are formed wherever there is lymphoid tissue, and cells in all stages of development are found in these tissues.

LIVER

During fetal life the liver is actively engaged in the formation of blood cells, particularly from the second to the fifth month, but at the time of birth this function has almost completely disappeared, although active blood islands have been found in the liver of premature and full-term infants up to the fifteenth day of life. Like the spleen, however, the liver possesses, through its reticulo-endothelial tissues, the potential ability to produce blood cells and in certain diseases may revert to this fetal function. This happens, for example, in the myeloid metaplasia observed in myelogenous leukemia and in the extramedullary erythropoiesis found in erythroblastoses. The liver is rich in reticulo-endothelial cells (Kupffer's cells), which react in the same manner as do reticulo-endothelial cells elsewhere in the body. A few blood monocytes may be formed in the liver.

Bilirubin is the chief pigment in human bile and is derived from the hemoglobin of the erythrocytes so that any condition which results in an increased destruction of red blood cells causes a greater production of this bile pigment. This pigment is formed throughout the body by the cells of the reticulo-endothelial system and normally there is from 0.2 to 0.8 mg. of bilirubin per hundred cubic centimeters of blood serum. The transformation of hemoglobin to bilirubin is an intracellular process within the reticulo-endothelial cells. The secretory cells of the liver merely excrete the pigment that reaches them preformed and do not play any part in its formation. Diseases in which excessive destruction of erythrocytes is a prominent feature throw an extra burden on the liver.

In addition to the function of the reticulo-endothelial portion of the liver and its excretion of pigments, the liver has a regulatory effect on erythropoiesis which is due to the maturation factor of Castle which is stored within the organ. This material is apparently formed in the stomach but after absorption is stored in the liver from which site it is liberated to the blood stream. By this means the liver is essential for erythropoiesis and maintains a regulatory effect on the bone marrow.

The liver is the principal site of formation of fibrinogen, as well as the source of prothrombin, so that the organ is of the greatest importance in relation to blood coagulation. The relationship between the prothrombin content of the blood and liver function is so intimate that the response of the prothrombin level to vitamin K administration serves as a liver function test.

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THE ERYTHROCYTES

MANY CONTROVERSIAL POINTS ARISE IN A DISCUSSION OF THE ORIGIN AND development of the various types of blood cells. Although these are of importance for complete understanding of hematopoiesis, they are not essential for diagnosis and treatment of blood dyscrasias, and, for the student, a complete consideration of all phases of the problem is likely to be more confusing than enlightening. This discussion, therefore, will be limited to essential features, and certain controversial points will be passed over rather lightly.

The earliest blood cells to appear in the embryo are erythrocytes which arise from mesenchymal tissue of the yolk sac. These are nucleated but as time goes on they are replaced by non-nucleated erythrocytes arising from the reticulo-endothelial tissues. The leukocytes appear in the circulating blood at a considerably later stage of embryonic development. They arise from reticulo-endothelial cells but are not numerous until fetal hematopoiesis shifts to the bone marrow about the third month. The formation of blood cells in the adult is a function of the reticulo-endothelial tissues. Erythrocytes and granulocytes are formed in the bone marrow, lymphocytes, in the lymphoid tissues, and monocytes, from reticulo-endothelial cells in various locations. The point of greatest controversy concerns the question of whether all blood cells are derived from a single primitive stem cell in the reticulo-endothelial system or whether each series of cells has an individual precursor which gives rise to only one type of cell.

According to the monophyletic theory there is a single polyvalent cell in the reticulo-endothelial tissues which is capable of producing, under varying stimuli or environmental conditions, any of the types of cells found in the blood stream. This cell is called a hemocytoblast or lymphoidocyte, and from it are derived erythrocytes, granulocytes, lymphocytes, and monocytes.

The polyphyletic theory assumes that a separate stem cell is present for each series. This concept is modified by those who believe that there are two

primitive stem cells (rather than a separate precursor for each series), one giving rise to erythrocytes and the other to all forms of leukocytes. According to the most widely held theory, each series of cells arises from a separate stem cell in the reticulo-endothelial system. These cells develop into erythrocytes, granulocytes, lymphocytes, and monocytes, respectively. As it is generally agreed that the primitive stem cell or cells are derived from fixed reticular cells of the reticulo-endothelial tissue, the controversy is mainly concerned with whether or not there is another intermediary primitive cell.

STRUCTURE AND FUNCTION OF THE ERYTHROCYTE

The normal mature erythrocyte is a circular biconcave disk definitely thicker at the periphery than in the central portion. There is no demonstrable internal structure and no nucleus. Although a cell membrane cannot be demonstrated, the reaction of the cell to hypotonic and hypertonic saline solutions indicates that the outer surface of the cell acts as a membrane even though no anatomic membranous cover can be observed. A large proportion of the cell—the functioning portion—is made up of hemoglobin. This is presumably held either in the form of a gel within the meshes of a spongelike stroma of lipoid substance or intimately bound to this substance.

The cells are extremely elastic. They are capable of undergoing great distortion when passing through small capillaries and can withstand great trauma without breaking. The peculiar configuration of erythrocytes, which gives them a large surface area in comparison with cell volume, facilitates absorption and discharge of oxygen. It has been estimated that the total sur-

PLATE I THE ERYTHROCYTIC SERIES

1. Megaloblast. This immature cell is similar to the myeloblast and lymphoblast in its structural characteristics, but the chromatin structure of the nucleus tends to be somewhat coarser and the cytoplasm more opaque.
2. Megaloblast with a still more opaque cytoplasm and a more dense nuclear structure.
- 3, 4. Erythroblasts showing dense blocks and strands of chromatin in the nucleus.
5. Normoblast with Howell-Jolly bodies.
6. Erythrocyte with Howell-Jolly body.
7. Normoblast with polychromatophilic cytoplasm.
- 8, 9, 10. Normoblasts.
11. Polychromatophilic erythrocyte.
- 12, 13. Reticulocytes.
14. Four normal erythrocytes.

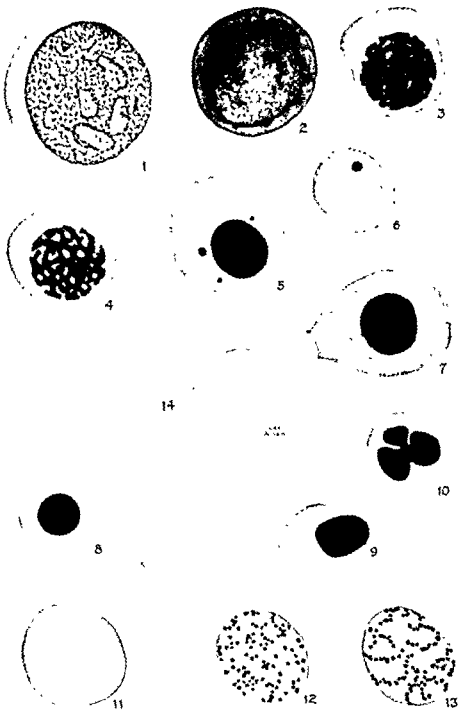


PLATE I

face area of all the erythrocytes in the body is in the neighborhood of 3000 square meters, or 1500 times the surface area of the body.

The primary function of erythrocytes is to transport oxygen from the lungs to the tissues and this depends entirely on their hemoglobin content. While the blood is within the lung capillaries where oxygen tension is high, oxygen combines chemically with hemoglobin to form oxyhemoglobin, but when the blood reaches the capillaries of tissues where oxygen tension is low, the oxyhemoglobin, an unstable compound, undergoes dissociation and oxygen is liberated to the tissue fluids. The reduced hemoglobin is then carried back to the lungs for reoxygenation. Carbon dioxide is carried from the tissues to the lungs in solution and not in chemical combination with the hemoglobin. The alteration in the acidity of the blood resulting from the change of oxyhemoglobin (HbO_2) to reduced hemoglobin (HbO) facilitates transportation of carbon dioxide. This change in acidity helps to maintain a normal acid-base equilibrium.

DEVELOPMENT OF ERYTHROCYTES

The erythrocytes that appear in the embryo originate in that portion of the primitive mesoderm which gives rise to the vascular system. They collect into syncytial masses and cords which liquefy at the center. After liquefaction takes place, certain cells break loose from their surroundings and begin to circulate through the vascular channels. At a later stage of fetal life, formation of the erythrocytes occurs primarily in the liver and spleen but it finally becomes established in the bone marrow. As this function is taken over by the bone marrow, erythropoiesis decreases in the liver and spleen so that at the time of birth all these cells are being formed in the bone marrow. With advancing age less and less of the bone marrow retains its erythropoietic function; in adult life the erythrocytes are formed primarily in the flat bones, such as the sternum, ribs, and pelvis, and in the proximal ends of the long bones as indicated in Figure 2.

The erythrocytes are believed by many to develop intravascularly, arising from endothelial-like cells which line the capillaries. These cells divide, the daughter cells becoming the primitive erythrocytes and the parent cells remaining in the capillary wall. The daughter cells progress to the stage of mature erythrocytes and are then ready for duty in the circulating blood. Isaacs believes that the cells develop in a sticky, jelly-like matrix which liquefies and frees the cells into the circulation upon their maturity. Doan and others believe that portions of the capillary bed are intermittently shut off from the circulation, either completely or partially, and that in these areas the

circulation is so slow that the oxygen tension becomes lowered to a point at which it serves as a stimulus to erythrocytic production. In these areas the cells develop to maturity. When they are ready for the peripheral circulation, there is a relaxation of the capillary constriction, with resumption of blood flow through that portion of the capillary system in which the cells were developing, and they are carried away by the blood stream. Others believe that the erythrocytes, like the granulocytes, arise from reticulo-endothelial cells outside the capillaries and upon maturity gain access to the circulation.

The mechanism whereby the number of erythrocytes in the blood is maintained at a constant level is not known, although many factors which influence development and maturation of erythrocytes are recognized. The red cells are apparently destroyed by the reticulo-endothelial tissues, with the spleen playing the most important role, and there is some evidence to show that the products derived from this cellular destruction act as a stimulus to erythropoiesis.

There are many substances necessary for proper red cell formation but many of the details and the exact role of certain of the factors are not clear. The anti-anemic or maturation factor of Castle is essential for proper growth and development of the cells but its exact nature and composition are unknown. In its absence an anemia develops and it is undoubtedly an important regulatory factor in maintaining normal erythropoiesis. In order that this maturation factor may be produced it is necessary that the intrinsic factor in the gastric content be present and also that the extrinsic factor be obtained from foods. The recent studies on folic acid show that although folic acid is neither the extrinsic nor intrinsic factor it is necessary for normal erythropoiesis and in its absence from the diet, or because of improper absorption, a macrocytic anemia develops.

Folic acid is a member of the vitamin B group and other members of this group such as pyridoxine, riboflavin, niacin, and others appear to play some part in erythropoiesis. Their exact role is unknown but their absence from the diet of experimental animals causes anemia. Vitamin C may also be necessary but again its exact role has not been determined.

Iron is necessary for hemoglobin formation and copper, although it does not enter into the hemoglobin molecule, is needed as a catalytic agent. An adequate protein intake is necessary and an anemia develops in its absence but globin apparently can be formed even though the protein and amino-acid intake is exceedingly low. The pituitary gland has been shown to exert some regulatory effect on hematopoiesis but its mode of action is in doubt. The thyroid is apparently not directly concerned with hematopoiesis but an absence of its secretion slows down the development of cells.

Low oxygen tension in the air and in the alveoli of the lungs stimulates the production of erythrocytes and anoxia of the hematopoietic centers may be an important factor in maintaining a normal erythrocyte level.

MATURATION OF ERYTHROCYTES

The terminology used for the various stages of erythropoietic development is confusing and variable and there is no agreement among workers in the field of blood diseases as to the exact progress of maturation of the erythrocytes. There are two schools of thought on erythropoiesis. One group believes that there are two separate developmental series of erythrocytes, the normal erythrocyte developing through the hemocytoblast, proerythroblast, and normoblast stages into a normal cell, while the megaloblast is found only in fetal marrow or in the marrow of pernicious anemia and the related macrocytic anemias. The megaloblast develops through several stages into an erythrocyte which is larger than normal. Naegeli, Downey, Jones, Wintrobe, and others adhere to this view. The second school of thought, including Sabin, Doan, Isaacs, and others, believes that the megaloblast is merely an early stage in the development of a normal erythrocyte and points out that, although megaloblasts are numerous in the bone marrow of patients with pernicious anemia, they are also present in normal marrow as precursors of normal erythrocytes. Isaacs found 60,000 to 112,000 megaloblasts per cubic millimeter of bone marrow taken from patients with pernicious anemia whereas there were 25,000 to 35,000 per cubic millimeter in normal marrow. The author agrees with the latter concept—that the megaloblast is an immature cell in the normal development of the erythrocyte. He has been unable to distinguish to his own satisfaction and unable to demonstrate to students the morphologic differences between the megaloblast and the early pronormoblast, so that cells indistinguishable from the megaloblast are encountered in normal marrow. This concept also seems to present a more logical explanation for the bone marrow findings in pernicious anemia in which there appears to be a maturation arrest at the megaloblast level, with further development occurring when adequate therapy is given so that the megaloblasts mature and consequently diminish in number in the marrow.

The following terminology and concept of development will be subject to criticism from many sources.

Megaloblast

The megaloblast is the earliest form of erythrocyte that can be recognized by its structure. It is a large cell, averaging 12 to 18 microns in diameter. The

nucleus is round or oval, usually centrally placed, and occupies from one-half to two-thirds of the cell body. A network of fine chromatin threads is evenly distributed throughout the nucleus. Because of the fine threadlike network and the even distribution, the nucleus stains a light purple or lavender with Wright's stain. It may contain from two to eight nucleoli. Nucleoli are small round light-staining areas which have a bluish tint and are surrounded by a denser rim of chromatin material. The cytoplasm of the cell is nongranular and deeply basophilic; it takes a deeper blue stain than the blast cells of other series. On a blood smear this cell has a thick appearance, and the dark blue staining reaction of the cytoplasm extends to the periphery of the cell. The cell does not contain hemoglobin.

Erythroblast

In the process of maturation the immature erythrocyte and its nucleus become smaller, the chromatin in the nucleus becomes more densely packed, and when hemoglobin appears in the cytoplasm, it acquires a slightly eosinophilic staining reaction. In the erythroblast stage the cell is considerably smaller than in the megaloblast stage, and the nucleus is correspondingly reduced in size. The evenly distributed chromatin material becomes compressed into deeply staining blocks. These stain a deep purple, are irregular in size and shape and sharply demarcated, and have irregularly shaped clear spaces between them. When grouped about the periphery of the nucleus, they suggest a clockface or cartwheel nucleus. This blocking of the chromatin into sharp, cleaneut masses is so characteristic that there is little danger of mistaking the erythroblast for any other type of bone marrow cell. The nucleus is usually round and centrally placed. The cytoplasm is gaining its complement of hemoglobin; it has lost the deep blue color and owing to the increasing eosinophilic staining reaction becomes bluish gray or slate gray. In some cells the cytoplasm approaches even closer the color of the normal erythrocyte, staining a pink or buff color.

Normoblast

The normoblast is the type of nucleated erythrocyte most commonly encountered in peripheral blood. It is the most mature of the nucleated forms, being but slightly larger than the normal non-nucleated erythrocyte. The nucleus has become small and pyknotic and is dense and uniform in its staining reaction, so dense that no details of the chromatin structure can be made out. It is a deep bluish purple in color. The nucleus is usually round and centrally placed, but occasionally it may be at one side or may even

appear to be partially extruded from the cell. In some instances it may be lobulated with two, three, or four small rounded lobules, which gives it a clover leaf appearance. The cytoplasm contains its normal complement of hemoglobin and consequently has the same staining reaction as the normal mature erythrocyte. In some of the cells the cytoplasm has a slightly bluish cast, a diffuse basophilia or polychromatophilia, which is also encountered in some young non-nucleated erythrocytes. Bluish dots (basophilic stippling) or even a fine bluish network may occasionally be observed in cytoplasm of normoblasts with ordinary staining technic.

The next stage in the development of the erythrocyte is loss of the nucleus and entry of the cell into the peripheral circulation.

DESTRUCTION OF ERYTHROCYTES

The life span of an erythrocyte and the exact method by which it is removed from the blood stream are not known. Phagocytosis of the cells, or of parts of the cells after disintegration, occurs in the reticulo-endothelial tissues, especially in those of the spleen and bone marrow, and it is thought that this is the principal method of removal. The cells are subjected to great mechanical trauma in the vascular system, both from the force of the blood stream as it leaves the heart and from the changes in shape produced during their passage through the smaller channels; there is also chemical trauma from constant alteration in the osmotic tension. As a result of these mechanical and chemical insults the cells may be broken up within the blood stream and the particles removed, or the surface membrane may be altered in such a way that entire cells are phagocytosed by the cells of the reticulo-endothelial system. The organ most actively engaged in the removal of these cells is the spleen. There is little evidence to support the theory that erythrocytes are withdrawn from the circulation by hemolysis.

After the cell has been removed from the circulation by the reticulo-endothelial tissue, the pigment portion is separated from the stroma. The stroma is not retained by the body, it is probably catabolized as an ordinary protein. The iron-containing portion of the pigment is held, at first by the reticulo-endothelial cells wherever the destruction of the cell occurs. Ultimately it finds its way to the bone marrow, where it is reutilized in the production of new hemoglobin. The non-iron-containing pigment of hemoglobin is converted to bilirubin and excreted by the liver.

Since the erythrocytes are non-nucleated cells and are incapable of growth, reproduction, or repair, their life expectancy is considerably shorter than

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Anisocytosis

The size of the erythrocytes varies in different types of anemia: In some anemias, the small cells or microcytes predominate; in others, large cells or macrocytes. In many cases of long-standing anemia there is a wide difference between the size of the largest and the smallest cells to be seen on a smear. The difference is most prominent in pernicious anemia. In normal blood the size of the cells tends to be uniform.

Poikilocytosis

Moderate variation in the shape of the erythrocytes is observed in many cases of chronic anemia. It is most pronounced in sickle cell anemia, in which the characteristic feature is the presence of extreme poikilocytosis with a predominance of fusiform, sickle, crescent- or oat-shaped cells. Great variations in the shape of the erythrocytes are also encountered in pernicious anemia, in which the abnormal cells assume many irregular and bizarre forms. Such distortions are inherent in the cells; they are not due to mechanical trauma produced while making the smear.

Variations in Staining Reactions

Variations in the staining reaction of erythrocytes are frequent. The simplest and most commonly encountered alteration is achromia or hypochromia, in which the cell is pale and has an excessively large central pale area. In many cases the enlargement of the central area is so extreme that only a narrow rim of cytoplasm remains at the periphery of the cell (ring or pessary form). When this peripheral ring of cytoplasm is also stained faintly, the cell is spoken of as a "ghost" cell. Since other factors besides the amount of hemoglobin influence the depth of the staining reaction, neither the degree of anemia nor the hemoglobin content can be judged accurately from the appearance of the erythrocytes on the smear. Two smears from the same patient and, in fact, different areas on the same smear may show considerable difference in the depth of staining. Hypochromia is commonly associated with microcytosis.

Hyperchromia indicates increased intensity of the staining reaction and is associated with decrease in size or absence of the central pale area. This change in the appearance of the central area is probably due to greater thickness of the cell rather than to actual increase in the amount of hemoglobin per unit of cytoplasm. Hyperchromia is practically always associated with macrocytosis.

that of ordinary tissue cells. Bile pigments are derived almost entirely from destroyed erythrocytes, and chemical determinations of the amount of such pigments excreted in a given period of time by a normal subject have suggested that the life span of the erythrocyte is about three weeks. Recent investigations, however, have shown that at least some of the cells persist in the circulation for periods up to 120 days. These data were obtained by transfusion experiments in which the transfused corpuscles were identified in the recipient's blood stream by means of their specific agglutinogens. The average duration of life for an erythrocyte is probably somewhere between these two extremes, but it is undoubtedly longer than three weeks.

ERYTHROCYTES ON THE BLOOD SMEAR

Erythrocytes as they are found in the peripheral circulation are biconcave disks with an average diameter of about 7.5 microns, a thickness at the periphery of about 2.2 microns, and an average cell volume of about 87 cubic microns (Fig. 3). On a well made smear with the cells lying flat and only one



FIG 3. Diagrammatic sketch to illustrate the size and shape of the normal erythrocyte layer deep, the erythrocytes stain more deeply at the periphery and gradually shade off to a pale central area that is the thinnest portion of the cell. Microscopic examination of the cells may reveal certain variations from the normal appearance. These are

1. Variations in size (anisocytosis)
 - Microcyte—less than 6 microns in diameter
 - Macrocyte—over 10 microns in diameter
2. Variations in shape (poikilocytosis)
3. Variations in staining reactions
 - Achromia or hypochromia
 - Hyperchromia
 - Polychromatophilia or polychromasia
 - Punctate basophilia or basophilic stippling
4. Variations in structure
 - Cabot's rings
 - Howell-Jolly bodies
 - Reticulocytes
 - Nucleated erythrocytes

The Erythrocytes

Target Cells

Target cells are erythrocytes with a rounded central area, rather stained; a clear ring surrounding this, lightly stained; and a dense cytoplasm about the periphery of the cell.

Ovalocytes

Oval- or elliptic-shaped erythrocytes are infrequently encountered in a normal blood smear. In some persons these ovalocytes predominate,



FIG 4 Photomicrographs of erythrocytes. In the lower right are cells with How bodies. The other plates show normoblasts.

cent or more of the erythrocytes having this shape. Such a condition is called ovalocytosis.

Spherocytes

Spherocytes are erythrocytes that have lost their normal biconcave shape and become nearly spherical. Since their thickness is increased while their diameter is lessened, their volume remains about normal. Such cells are more easily hemolyzed than are normal cells. Spherocytosis, a predominance of these cells, is found in familial hemolytic icterus.

Polychromatophilia, polychromasia, and diffuse basophilia are synonymous terms indicating a faint diffuse bluish tint in the cytoplasm of the erythrocytes. If the diluted Wright's stain is too alkaline, a bluish cast will be imparted to all the erythrocytes; this should not be confused with polychromatophilia, in which only certain cells (usually macrocytes) show the bluish gray color, while the neighboring erythrocytes have a normal staining reaction. Polychromatophilia is interpreted as evidence of immaturity of the cell. When, therefore, it is found in the peripheral blood, it is an indication of active erythropoiesis in the bone marrow. It is frequently seen in the cytoplasm of normoblasts and is always present at some stage in the development of erythrocytes.

Punctate basophilia, or basophilic stippling, appears as small pinpoint dots of basophilic material scattered through the otherwise normal cytoplasm of an erythrocyte. This staining reaction may occur under the same circumstances as does diffuse basophilia. It may also appear in the cytoplasm of nucleated forms of erythrocytes. It is particularly common in the blood of patients with chronic lead poisoning and thus provides a valuable sign in the differential diagnosis of this type of intoxication. However, the fact that it occurs under such circumstances casts doubt on the significance of basophilic stippling as specific evidence of immaturity of the cell, being more suggestive of degenerative change in a young erythrocyte. All cells that react to Wright's stain with basophilic stippling can be shown to be reticulocytes when stained with brilliant cresyl blue, but not all reticulocytes are stippled.

Variations in Structure

All erythrocytes are originally nucleated. The nucleus usually disintegrates or is extruded before the cell enters the circulation, but in occasional instances remnants are retained. There are two rare types of nuclear remnants, which are more frequently encountered in the blood of patients with pernicious anemia than in any other condition. Howell-Jolly bodies are small, rounded, densely staining particles of nuclear material. They usually occur singly although occasionally there may be several within one cell (Fig. 4). They are likely to be seen near the periphery of the cell. Occasionally they are found in nucleated erythrocytes with an apparently intact nucleus. Cabot's rings are circular or figure-of-eight-shaped strands of basophilic material which probably represent remnants of nuclear membrane.

Significance of Reticulocyte Counts

The consensus is that the presence of reticulocytes in the blood stream indicates active erythropoiesis in the bone marrow. The reticulocyte count is of importance under the following conditions:

1. In pernicious anemia, the effectiveness of administration of liver extract is indicated by increase in the number of reticulocytes before any change is apparent in the hemoglobin level or in the erythrocyte count. A therapeutic trial of liver extract is of value as a diagnostic procedure in cases in which the presence of pernicious anemia is questionable. In known cases of pernicious anemia the potency of a liver preparation can be tested by ascertaining the degree of reticulocyte response.

2. In iron deficiency anemias, the number of reticulocytes rises in response to administration of adequate amounts of iron.

3. In anemias of the hemolytic type, particularly familial hemolytic icterus, there is a spontaneously high reticulocyte count.

4. Following acute hemorrhage there is a rise in the number of reticulocytes during the period of blood regeneration.

5. Reticulocytes are more numerous immediately after splenectomy.

6. Reticulocytes are fewer in anemias due to aplasia or hypoplasia of the bone marrow. A reticulocyte count is of value in distinguishing between aplastic anemia, in which there are fewer reticulocytes, and idiopathic thrombopenic purpura or agranulocytosis, in which their number is normal or increased.

7. Repeated transfusions may lead to diminution in the number of reticulocytes.

HEMOGLOBIN

Hemoglobin is the coloring matter of the erythrocytes. By means of this pigment the red cells are able to perform their function of transporting oxygen to the tissues, the hemoglobin forming a loose combination with oxygen in the lungs and releasing it to the tissues. Hemoglobin is a conjugated protein consisting of an iron-containing pigment and a protein, "globin." The iron-containing portion consists of a porphyrin molecule composed of four pyrrole nuclei. The porphyrins are pigments which form the basis for many complex compounds in plant and animal life and are capable of combining with various metals. Protoporphyrin when combined with iron, probably in the ferrous state, forms the iron-porphyrin compound of hemoglobin, and is called "heme." When heme is combined with a

Reticulocytes

Reticulocytes are not evident on fixed smears stained by the ordinary methods. With a vital staining technic whereby the cells are stained while in a living state, basophilic granules or filaments can be observed in the cytoplasm of 0.2 to 0.8 per cent of erythrocytes. Brilliant cresyl blue, the stain most commonly used for demonstrating reticulocytes, stains the reticulum deep blue. The amount and arrangement of this granulo-filamentous substance vary in different cells: There may be only a few granules or threads of material scattered through the cytoplasm; or granules and filaments may

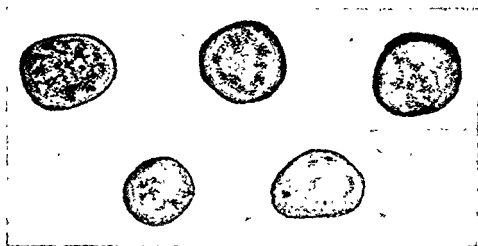


FIG. 5. Reticulocytes showing varying amounts and arrangements of the reticular substance. The lower cells, which have smaller amounts of the reticular material, are presumably more mature (Hal Downey, *Handbook of Hematology*. Paul B Hoeber, Inc.)

combine to form a diffuse network throughout the entire cell. In some instances a rather dense wreathlike arrangement is found near the periphery. Reticulocytes are slightly larger than normal erythrocytes. Some of them are polychromatophilic with Wright's stain. The amount of reticulum present has been considered to be an indication of the age of the cell; the younger the reticulocyte, the more reticulum it contains (Fig. 5).

The nature of the reticulum is not known, but apparently it is not composed of either nuclear chromatin or mitochondria. It has been generally conceded that reticulocytes represent a slightly immature stage of erythrocytes, but certain features of their occurrence cast some doubt on this hypothesis. It must be acknowledged, however, that they represent a stage in the maturation of erythrocytes and that they afford the most reliable criterion for estimating erythropoietic activity of the bone marrow.

as is any protein, that is, it is converted to amino acids and then reutilized. The iron-containing portion is broken down, liberating iron, which is stored in the liver and spleen for reutilization, and non-iron-containing pigment, which is converted to bilirubin. The latter is brought to the liver and excreted, forming the principal if not the entire source of bile pigment. After excretion into the intestine, the bile pigments are converted by bacterial action into urobilinogen, of which part is excreted and part reabsorbed.

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protein, the compound is called "hemochromogen"; when the protein with which it combines is globin, the resulting hemochromogen is hemoglobin. Hemoglobin is therefore iron + porphyrin + globin. The pigment portion of hemoglobin constitutes about 4 per cent and the globin about 96 per cent of the hemoglobin molecule. Iron comprises 0.335 per cent.

Hemoglobin combines with oxygen in the proportion of two atoms of oxygen to one atom of iron. Therefore 1 Gm. of hemoglobin unites with 1.34 cc. of oxygen. Normally arterial blood is 94 to 96 per cent saturated with oxygen; venous blood is 60 to 85 per cent saturated. The combination with oxygen is unstable because the globin allows oxygenation of the hemoglobin to oxyhemoglobin but prevents oxidation. Methemoglobin is a true oxide, one atom of oxygen combining with one atom of iron. This combination is stable and not reversible. It occurs in poisoning by certain drugs such as nitrates, chlorates, acetanilid, and nitrobenzene. Carbon monoxide combines with hemoglobin in the same proportion as does oxygen but forms a stable compound which is not easily disrupted; consequently the hemoglobin is no longer available to carry oxygen. Because of its ability to displace oxygen, inhalation of carbon monoxide is highly dangerous to life.

Although the constituents of hemoglobin are known, the method of its synthesis within the body remains a mystery. The pigment portion, heme (porphyrin + iron), is present in a vast majority of all foodstuffs, and it is possible that the food porphyrins are broken down to their pyrrole constituents and resynthesized. Globin is available in most meats, its absence may be a definite factor in limiting hemoglobin production. Iron is absorbed from the upper portion of the gastrointestinal tract, probably in the ferrous form, and is transported either to the liver, spleen, and other parts of the reticulo-endothelial tissues for storage or to the bone marrow for utilization in hemoglobin. Just how the various constituents are assembled is not known.

Hemoglobin first appears in the cytoplasm of the erythrocyte at a relatively early stage in the cell's development. With its first appearance a slight eosinophilic staining reaction is noted in the cytoplasm close to the nucleus of the cell. As the hemoglobin increases in amount, the cytoplasm becomes diffusely polychromatophilic, and with further increase in hemoglobin the early basophilia decreases and the pink eosinophilic staining reaction of the mature cell prevails.

When erythrocytes are removed from the circulation by reticulo-endothelial cells, the hemoglobin is broken down into globin and iron-containing pigment. The globin is probably handled in the same manner

Myeloblast

The myeloblast, normally found only in the bone marrow, is usually large, ranging from 15 to 20 microns in diameter. In some instances, however, it is so small that the term *micromyeloblast* has been applied to it. The ordinary large myeloblast when stained with a polychrome dye has a large nucleus which is round or oval in shape and stains a light reddish purple. The fine and evenly distributed strands of chromatin form a delicately woven network which contains from three to five nucleoli. The nuclear structure of the myeloblast gives it a bland and homogeneous appearance with no apparent nuclear membrane. The nucleus is frequently eccentrically placed.

The cytoplasm of the myeloblast is abundant, is light blue in color, and lacks both a perinuclear clear zone and granules. In the absence of specific granules in the cytoplasm it is impossible to tell whether a particular myeloblast will develop into a neutrophilic, eosinophilic, or basophilic myelocyte.

Myeloblasts are common in the bone marrow but only under unusual conditions, such as myelogenous leukemia, do they appear in the peripheral blood stream. Their structure is so similar to that of the primitive cells of other series that it is difficult to separate them with certainty from lymphoblasts. The lymphoblast may have a coarser chromatin network, fewer nucleoli in the nucleus, and a perinuclear clear zone in the cytoplasm, but neither cell contains cytoplasmic granules. The differentiation of these cells in the peripheral blood is accomplished primarily by noting the type of mature cell that predominates on the smear. If the predominating mature cell is a lymphocyte, it is probable that the immature cell in question is a lymphoblast; if myelocytes or mature granulocytes predominate, the cell in question is probably a myeloblast. Thus the immature cell is recognized by the company it keeps.

The next stage of development is manifested by the appearance of a few purplish red, peroxidase-positive granules in the cytoplasm. These ultimately develop into the characteristic neutrophilic, eosinophilic, or basophilic granulations of the myelocyte, but at first appearance they are nonspecific. This stage, the premyelocyte, is the immediate precursor of the myelocyte. It is, however, merely an intermediary step in development, and does not merit further discussion here. It has been our policy in teaching medical students to classify as myeloblasts those cells of the series which do not contain granules, and after granulations appear to classify them as myelocytes. This

THE LEUKOCYTES

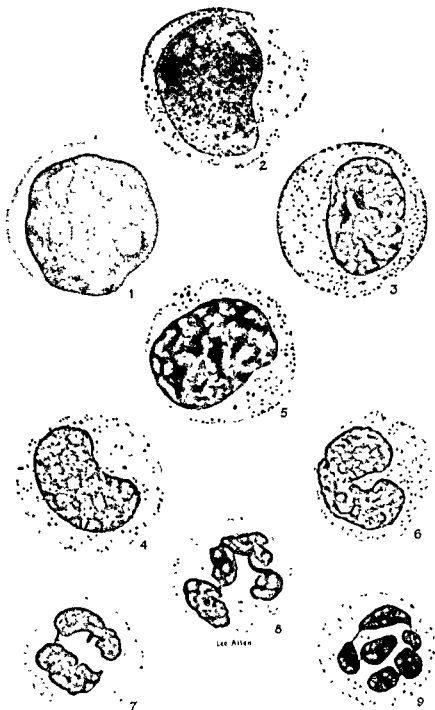
I. THE GRANULOCYTIC OR MYELOID SERIES OF CELLS

POLYMORPHONUCLEAR NEUTROPHILIC LEUKOCYTES

POLYMORPHONUCLEAR NEUTROPHILIC LEUKOCYTES, IN THEIR MATURE FORM, are commonly spoken of as neutrophils or "polys." Under normal conditions they comprise about two-thirds of the white blood cells in the circulation. Developmentally, they are end cells and when damaged are incapable of reproduction or regeneration. They are capable of active ameboid motion and are phagocytic. Their primary function is to combat infections. Although many conditions cause an increase in the number of these cells in the blood stream, the greatest response occurs with a pyogenic infection. The mechanism whereby this and other conditions bring about an increased production of cells is not known.

The life of a neutrophil is short, apparently from two to five days under normal conditions. Many of the cells are lost or destroyed in fulfilling their protective mission; most of those not destroyed in this manner are excreted through the gastrointestinal tract. Appreciable numbers are eliminated in the saliva, and a similar eliminative process takes place throughout the entire gastrointestinal tract. Many other cells are eliminated through the respiratory and nasal mucous membranes.

The granulocytic cells originate in the bone marrow in the same areas where erythrocytes develop but are produced extravascularly rather than within the capillaries. They are derived from a fixed reticular cell of the reticulo-endothelial system. This gives rise to a primitive free cell which is the direct precursor of the myeloblast. The myeloblast is the earliest cell of this series to be recognized morphologically by ordinary staining technique.



simplifies the discussion of maturation and is satisfactory for all practical purposes.

In place of the usual type of granule which appears at this stage of the cell's development there may be one or several small eosinophilic rodlike structures in the cytoplasm, the so-called Auer bodies. Cells containing these bodies are encountered most frequently in acute forms of myelogenous leukemia.

Neutrophilic Myelocyte

The neutrophilic myelocyte is of approximately the same size as the myeloblast, averaging from 12 to 20 microns in diameter. The nucleus is large, and round or oval in shape but is frequently flattened on the medial side and eccentrically placed. The chromatin strands are fine and evenly distributed so that the nucleus is bland, light staining, and reddish purple in color and is similar to that of the myeloblast. Nucleoli are less numerous than in the blast cell stage, and the nucleus may be slightly smaller and may stain a little darker.

The cytoplasm is light blue in its staining reaction and contains the characteristic neutrophilic granules. In this stage the granules reach their greatest prominence. Their number depends upon the age of the cell. They may be few in number in the young forms; in the later stages there may be innumerable lavender or purplish granules. They are far more prominent than in the mature neutrophil, and the large, dark purple granules of the neutrophilic myelocyte must not be mistaken for the still larger and darker ones of the basophilic myelocyte. As the myelocyte becomes older the granules become smaller and less prominent.

PLATE II THE MYELOID OR GRANULOCYTIC SERIES—NEUTROPHILS

1. Myeloblast which does not contain specific granules 2. Myelocyte in which neutrophilic granules are present in the cytoplasm 3. Metamyelocyte in which the cytoplasm is changing from basophilic to eosinophilic 4. Metamyelocyte with basophilic cytoplasm but a relatively mature nuclear structure. 5. Metamyelocyte with eosinophilic cytoplasm but an immature type of nucleus. 6. Band, nonsegmented, or nonfilament neutrophil. 7, 8, 9. Mature segmented neutrophils.

In normal bone marrow the myelocyte is the predominant cell of the myeloid series. In myelogenous leukemia it appears in the peripheral blood stream in varying numbers. The more acute and rapidly progressive the disease, the higher the percentage of this immature cell. In many instances it is the predominant cell on the blood smear.

The bone marrow is more responsive in infants and young children than in adults and consequently reacts more vigorously to a given stimulus. For this reason neutrophilic myelocytes, occasionally found in the blood stream of children as a result of a severe infection, do not carry the same significance as they would in equal numbers in the blood of an adult. The response of the marrow in children to hemolytic and other types of severe anemia also results in a leukocytosis and the appearance of myelocytes in the blood stream. In some instances the reaction has been so marked that the term *pseudoleukemia* has been used when in reality the reaction was only secondary to a severe anemia.

Myelocytes appear in the blood stream of the adult less frequently than in that of children but they may occur not only as a result of leukemia but with an extreme bone marrow stimulation from a severe infection, occasionally with hemolytic anemias and metastatic malignant growths, and during recovery from agranulocytosis. The presence of myelocytes in the blood, however, should always suggest the possibility of myelogenous leukemia regardless of the absence of other typical features.

Metamyelocyte or Juvenile Cell

There are great structural changes in the cell between the myelocyte and the mature state. Development may progress in several ways so that it is difficult or impossible to give an accurate description covering all cells at this period of their growth. In the myelocyte the nucleus is large, light staining, and of uniform density whereas in the mature neutrophil it is small, with dense strands of chromatin. The cytoplasm is basophilic in the myelocytic stage but becomes eosinophilic in the mature cell. Progress through the intervening stages may be even and regular, or atypical forms may occur if either the nucleus or the cytoplasm matures at a more rapid rate than the rest of the cell.

In the normal process of development the metamyelocyte is considerably smaller than the myelocyte, ranging from 10 to 15 microns in diameter. The nucleus is smaller, the strands of chromatin are more dense and more deeply staining, the nucleoli have disappeared. The nucleus may be oval or kidney shaped. The cytoplasm has lost its basophilic tint and is now

the smear that the connecting strands are hidden and the nucleus appears rod shaped rather than segmented. The number of lobes in the nucleus of a neutrophil supposedly increases with the age of the cell, but this is not strictly true, the observer should pay more attention to nuclear structure than to nuclear segmentation in determining the age of the cell.

The cytoplasm is of uniform density with an eosinophilic staining reaction and is filled with fine light purple granules. These are small and evenly distributed throughout the cytoplasm and give a strongly positive peroxidase reaction. The living cell is actively motile.

In the presence of a severe toxic or metabolic disturbance the granules of the neutrophil may be larger, darker staining, and more prominent than normal, reverting to the type of granule found in the myelocytic stage. This condition is spoken of as "toxic granulation."

Vacuolation of the cytoplasm may occur and is also an evidence of degeneration. Cells showing excessive vacuolation may be particularly numerous in severe infections, and the ratio of cells showing this change to normal cells has been called the "degenerative index."

Arneth divided the neutrophils into a number of classes according to the number of lobes in the nucleus. When there is an increased production of these cells, more of the younger forms, including those with band nuclei, appear in the blood stream, when these comprise more than the normal 4 or 5 per cent, a nuclear "shift to the left" has occurred, which signifies a greater degree of immaturity of the cells than is normal. A "shift to the right" means an increased percentage of neutrophils having highly segmented nuclei and indicates that a majority of the cells are older than normal. A simpler scheme was introduced by Schilling in which the neutrophils are separated into only two groups (1) the segmented forms in which there are two or more lobes in the nucleus and (2) the younger cells with non-segmented nuclei (Fig. 6). This separation of the neutrophils into non-segmented and segmented forms should be a part of all differential counts.

Function of the Neutrophil

The neutrophilic leukocytes form the principal defense against bacteria which have invaded the body, in health they aid in preventing an invasion by these organisms. Tissue injury as well as bacteria apparently liberates a substance which attracts neutrophils so that they are the first cells to appear at the site of any chemical or mechanical damage to tissues as well as at the site of bacterial invasion. The neutrophil is phagocytic for many types of bacteria, the engulfed organisms being killed and destroyed. It is

eosinophilic in its staining reaction and contains neutrophilic granules which are smaller and stain less deeply than those of the myelocyte.

This typical course of maturation is altered in some cells so that the cytoplasm develops at a more rapid rate than the nucleus, and we find a cell with an immature nucleus which shows nucleoli but a mature eosinophilic cytoplasm with neutrophilic granulations. Not infrequently the cytoplasm is blotchy, and there are eosinophilic areas intermingled with the basophilic cytoplasm. In other instances the nucleus matures more rapidly than the rest of the cell, and we find an indented or band-shaped nucleus with rather dense chromatin strands surrounded by basophilic, immature cytoplasm containing granules like those of the myelocyte.

Band Neutrophil or Stab Cell

The band neutrophil is approaching maturity and is but slightly larger than the mature segmented neutrophil. The nucleus is elongated, of uniform width, and rounded at the ends. It is usually curved in a horseshoe or S shape and may show slight evidences of constriction at the points of bending. The chromatin is in heavy strands forming a coarse reticular network. It stains a light reddish purple. No nucleoli are present. The cytoplasm is eosinophilic and contains innumerable fine dustlike particles, which are the typical *neutrophilic granulations*.

These cells comprise about 4 or 5 per cent of the leukocytes in normal blood but are more numerous in any condition in which there is an increased production of neutrophils. An increase in the number of band neutrophils and the other young forms is called a "shift to the left." Since the nucleus is composed of only one lobe, the band neutrophil is called a "nonfilament" or nonsegmented cell.

Segmented Neutrophil

Segmented neutrophils are the mature neutrophils that normally comprise about 60 to 65 per cent of the white blood cells. As the cell matures it becomes smaller until at this stage its diameter is from 10 to 12 microns. The nucleus is smaller and more dense and pyknotic than in the immature stage. The chromatin strands are dense, the meshwork is tighter, and the chromatin tends to collect in irregular dense blocks which stain dark purple. The nucleus is lobulated, having from two to five lobes which are connected by single strands or filaments of chromatin material. Because of its nuclear configuration this cell is called a segmented or filament cell. The lobes vary in size, shape, and arrangement and may be so pushed together in preparing

one of the substances necessary for blood coagulation and it may aid in absorption from the intestinal tract.

EOSINOPHILS—POLYMORPHONUCLEAR EOSINOPHILIC LEUKOCYTES

The eosinophils, being members of the granulocytic or myeloid series of cells, originate in the bone marrow in the same areas in which neutrophils are formed. They arise from an extravascular fixed reticular cell and progress through the stage of a primitive free cell and myeloblast without the appearance of any cytoplasmic granules to distinguish them from other myeloid cells. Nonspecific azurophilic granules first appear in the premyelocyte stage of development, and the first differentiation of the eosinophil from the neutrophil and basophil occurs with the appearance of specific granulations in the myelocyte stage. The granules of the eosinophil are rounded bodies with sharply demarcated and well defined margins and are much larger than the granules of the neutrophil. The eosinophilic granules obtain their name from their great affinity for acid dyes. With the usual polychrome stain they take a brilliant red color.

Eosinophilic Myelocyte

The eosinophilic myelocyte is similar to the neutrophilic myelocyte in its structural characteristics except for the nature of the granules. The nucleus is large with a fine even chromatin network, and the cytoplasm is slightly basophilic in its staining reaction. The cytoplasm is filled with the large round red granules, which are frequently so numerous that they completely obscure it and may partially obscure the nucleus. The granules remain the same through all stages of the cell's development. Eosinophilic myelocytes are prominent in smears made from bone marrow and are proportionately more numerous in the marrow than are mature eosinophils in the peripheral blood.

Since the number of eosinophils is not increased with infectious diseases, this form of myelocyte does not appear in the blood stream as a result of severe infections, when found in the peripheral blood eosinophilic myelocytes are extremely suggestive of myelogenous leukemia.

Eosinophilic Metamyelocyte

The eosinophilic metamyelocyte conforms to the description of the neutrophilic metamyelocyte except for the eosinophilic cytoplasmic granules. It undergoes the same structural changes in the nucleus and cytoplasm during

not effective against all types of bacteria and is not phagocytic for tissue cells or cell fragments. It contains a proteolytic enzyme which aids in the digestion of necrotic tissue and the removal of fibrin of inflammatory origin. In these ways it is an important agent in the defense against invading or-

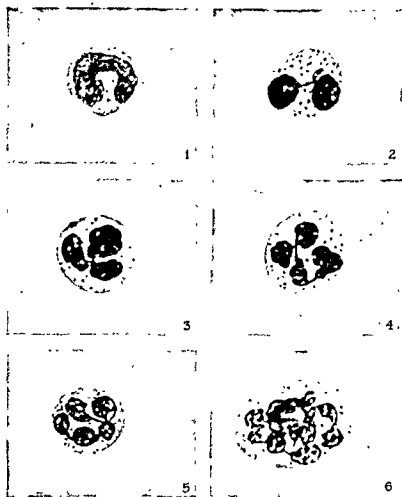


FIG. 6 Photomicrographs of polymorphonuclear neutrophilic leukocytes 1. Band, nonsegmented, or nonfilamented neutrophil 2 to 5. Showing two, three, four, and five lobed neutrophils. 6 Multilobulated neutrophil as found in a patient with pernicious anemia.

ganisms It also plays a part in preventing bacteria from gaining access to the body by the constant migration of cells to the surface of the mucous membranes where such organisms are present.

In addition to its part in the defense mechanism of the body, the functions of the neutrophil are vague and indefinite, but it may be a source of

one of the substances necessary for blood coagulation and it may aid in absorption from the intestinal tract.

EOSINOPHILS—POLYMORPHONUCLEAR EOSINOPHILIC LEUKOCYTES

The eosinophils, being members of the granulocytic or myeloid series of cells, originate in the bone marrow in the same areas in which neutrophils are formed. They arise from an extravascular fixed reticular cell and progress through the stage of a primitive free cell and myeloblast without the appearance of any cytoplasmic granules to distinguish them from other myeloid cells. Nonspecific azurophilic granules first appear in the premyelocyte stage of development, and the first differentiation of the eosinophil from the neutrophil and basophil occurs with the appearance of specific granulations in the myelocyte stage. The granules of the eosinophil are rounded bodies with sharply demarcated and well defined margins and are much larger than the granules of the neutrophil. The eosinophilic granules obtain their name from their great affinity for acid dyes. With the usual polychrome stain they take a brilliant red color.

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Eosinophilic Metamyelocyte

The eosinophilic metamyelocyte conforms to the description of the neutrophilic metamyelocyte except for the eosinophilic cytoplasmic granules. It undergoes the same structural changes in the nucleus and cytoplasm during

maturation. The granules are frequently so numerous and densely packed that they obscure the nucleus.

Eosinophil—Mature Form

Following the metamyelocyte stage the nucleus of the eosinophil becomes an elongated curved rod which ultimately divides into two or more lobes. A majority of the eosinophils encountered in the blood stream have two lobes although three-lobed nuclei are occasionally seen. These cells are more fragile than neutrophils and consequently are more easily broken in the process of making a smear. The cell membrane is frequently ruptured leaving the nucleus intact, the cytoplasm invisible, and the eosinophilic granules scattered about the nucleus. Such broken-up eosinophils should be counted as eosinophils in the differential count rather than classed as degenerated cells.

The nucleus of the intact cell is usually either a band or a two-lobed structure. It stains a light purple color and seldom becomes the small, dark, pyknotic, multilobulated nucleus commonly found in the neutrophil. The cytoplasm is eosinophilic but is usually completely hidden by the densely packed eosinophilic granules. Occasionally the granules are few in number and do not completely obscure the cytoplasm, but this is infrequent.

Eosinophils comprise from 2 to 4 per cent of the circulating leukocytes, unless they increase in number to 8 or 10 per cent, the increase is usually of no significance. They tend to disappear from the blood stream with acute infections, but a slight eosinophilia may occur during the convalescent stage from such infections.

The function of the eosinophil has not been determined with certainty although the cell has been shown to be phagocytic. The high iron content of the granules has led to speculation as to a possible role in iron metabolism, but this has not been confirmed. The extremely varied conditions that give rise to an eosinophilia make interpretation of function difficult. The cells

PLATE III. THE MYELOID OR GRANULOCYTIC SERIES—BASOPHILS AND EOSINOPHILS

1. Basophilic myelocyte. 2. Mature basophil 3. Eosinophilic myelocyte. 4. Mature eosinophil. 5. Eosinophil in which the cell membrane has ruptured.



Lee Allen



are increased in allergic diseases, parasitic infestations, some skin diseases, periarthritis nodosa, and in most cases of myelogenous leukemia as well as a few other conditions.

There is no definite relationship between local or tissue eosinophilia and an increased eosinophil count of the peripheral blood. Many lesions are characterized by a larger number of tissue eosinophils without the appearance of these cells in the blood stream. Local eosinophilic reactions are particularly frequent in lesions of the gastrointestinal tract and of the pleura whereas these lesions seldom cause an increased blood eosinophilia.

BASOPHILS—POLYMORPHONUCLEAR BASOPHILIC LEUKOCYTES

The basophils comprise the third type of granulocytic leukocytes found in the blood stream. They are formed in the bone marrow in the same manner and in the same location as the neutrophils and eosinophils. Their immediate precursor is the myeloblast, which does not contain specific granules, so that the first recognizable member of the series is the basophilic myelocyte.

The granules of the basophilic series of cells are large spherical or oval bodies, from 0.2 to 0.4 micron in diameter, which stain a very deep blue. The number of granules is variable. Usually they are sparsely but evenly scattered throughout the cytoplasm. Their size and appearance are the same in the immature and mature forms of the cell and they do not go through the maturation changes that occur in the neutrophilic granules.

Basophilic Myelocyte

The basophilic myelocyte is a large immature cell identical to the neutrophilic myelocyte except for the character of the basophilic granules, which are larger, more deeply staining, and usually less numerous than the neutrophilic granules at the same stage of development. The granules may occasionally be so numerous as to obscure the nuclear outlines and structure. These cells do not appear in the blood stream except with myelogenous leukemia. The cells go through the same stages of maturation as the neutrophilic myelocyte.

Band and Segmented Basophils

The description of the band and segmented neutrophil suffices for the basophilic type except for the granules. The nucleus seldom becomes extremely segmented, and nuclei with over two lobes are seldom encountered.

Because of this relative immaturity of the cell the nucleus is rather light staining and has a loose chromatin network. Broken or fragmented cells are seldom seen on the blood smear. The cytoplasm is similar to that of the neutrophil and contains varying numbers of the large deeply staining granules; occasionally only ten or twelve are present, but they are more numerous in some cells. They never completely fill the cell as is the case with eosinophils.

The basophils ordinarily comprise from 0.5 to 1 per cent of the leukocytes so that in many instances none are encountered in an ordinary differential count. These mast leukocytes or basophils have no relation to the tissue mast cells, and their function in the blood stream is unknown. In acute infections they tend to disappear completely from the blood stream and to reappear during convalescence.

They are occasionally more numerous in polycythemia vera, and in some cases of myelogenous leukemia they become very prominent. A slight increase in number has been noted in some types of skin disease and in some cases of cirrhosis of the liver but this is not constant.

II. LYMPHOCYTES

If the term *lymphocyte* is restricted to those forms which are found in the circulating blood and their immediate precursors in the fixed lymphatic tissues, these cells make their appearance late in embryonic development; but if the term is used to include the primitive hemocytoblast or lymphoidocyte, as is occasionally done, the cells are found very early in the yolk sac of the embryo. In postfetal life the lymphocytes are formed in all lymphoid tissues: in lymph nodes, spleen, tonsils, and the lymph nodules of the respiratory and gastrointestinal tracts. The cells arise from fixed and free reticular cells which give rise to the early primordial lymphoblast, which in turn divides through mitosis to more advanced forms of the cell. There are no definite steps in the development of lymphocytes, as is true in the granulocytic series, but there is a steady growth from the large, immature lymphoblasts to the small mature lymphocytes which comprise from 20 to 30 per cent of the white blood cells in the peripheral circulation under normal conditions (Fig. 7).

Lymphoblast

The lymphoblast is a large nongranular cell which measures from 15 to 20 microns in diameter on a fixed blood smear but is considerably smaller when

seen in tissue sections. Its structural characteristics are so similar to those of the myeloblast that the two cells ordinarily cannot be distinguished from

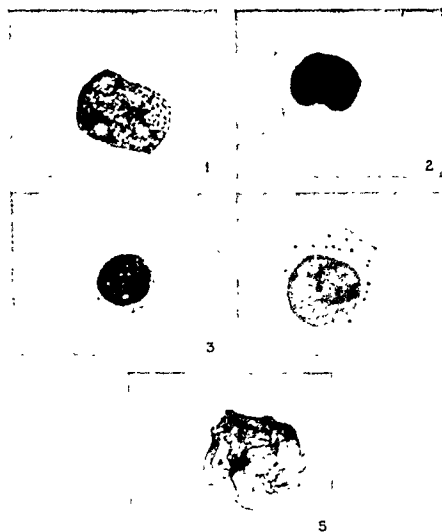


FIG. 7 Photomicrographs of lymphocytes 1. Lymphoblast with four nucleoli from a case of lymphocytic leukemia. 2 and 3 Normal lymphocytes 4 Large lymphocyte with azure granules in the cytoplasm. 5 Basket cell, a degenerated or broken-up lymphocyte in which only the nuclear material is visible

each other when seen on a blood smear and are recognized "by the company they keep." The nucleus is large, round or oval in shape, and occupies a large percentage of the cell area. The chromatin is arranged in strands

which are slightly coarser than those found in the nucleus of the myeloblast, and the chromatin network may be somewhat more dense at the periphery than at the center of the nucleus. In most of the cells, however, the network is uniform in its distribution and stains a light purplish color with polychrome dyes. Nucleoli are present in varying numbers.

The cytoplasm stains a light blue and does not contain granules nor does it give a peroxidase reaction. A perinuclear clear zone may be present. There are no definite development stages in the lymphocyte, but as the cell becomes older it becomes smaller and more deeply staining and nucleoli disappear from the nucleus.

Large Lymphocyte

The nucleus of the young lymphocyte is smaller than that of the lymphoblast and more darkly staining. It is round or oval in shape. The chromatin is dense but evenly distributed, and there is no definite interlacing network as in the young neutrophil or in the monocyte. This nuclear structure is one of the most important features in differentiating the lymphocyte from other cells on a fixed smear.

The cytoplasm is a clear hyaline blue and is usually abundant. In about 30 per cent of the cells granules are present in the cytoplasm. These are not numerous, are irregularly scattered throughout the cytoplasm, stain a red or reddish purple color, and are called "azure granules." They are larger in size and more distinctly outlined than the granules of the mature neutrophil but are smaller, darker, and more dense than those of the eosinophil. They do not give a peroxidase reaction. These granules are not evident in immature lymphocytes and are seldom encountered in the cells of lymphocytic leukemia.

PLATE IV. THE LYMPHOCYTIC SERIES

1. Lymphoblast.
- 2, 3 Large lymphocytes with azure granules.
4. Large lymphocyte without cytoplasmic granules.
- 5 Small lymphocyte with granules
- 6 Small lymphocyte without granules in the cytoplasm
7. Basket cell or degenerated cell. This represents the disintegrating nucleus of an immature lymphocyte
8. Naked nucleus or degenerated cell. This is also a disintegrating nucleus of a lymphocyte.



1



2



4



3

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5



7



6



8

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Mature Lymphocyte

The mature lymphocyte is from 6 to 10 microns in diameter and is characterized by having a nucleus which almost completely fills it. In the larger forms of the cell the cytoplasm is somewhat more abundant. In the smaller cells the nucleus stains a dark blue and is so dense that the nuclear structure cannot be determined and no chromatin network is evident. The distribution of the chromatin is not uniform, however, but tends to collect in ill defined clumps in which dense areas gradually shade off to give an "ocean wave" appearance. The clumps of chromatin are frequently more prominent at the periphery of the nucleus. In the larger forms of the cell the staining reaction of the nucleus is lighter, the chromatin is less dense, and the tendency to clumping is less evident. The nucleus is usually slightly indented on one side.

The cytoplasm of the mature lymphocyte is scanty and may be visible only at the point where the nucleus is indented or it may form a narrow rim which entirely surrounds the nucleus. In the larger cells it is considerably more abundant. Azure granules may be present and are commonly encountered within the nuclear indentation. The cytoplasm stains a light blue.

Abnormal Lymphocytes

Variations in the structure of the lymphocytes occur frequently. It is important to recognize them so that the abnormal lymphocyte is not confused with the immature lymphoblast. Many of the abnormal cells are large and have a great deal of cytoplasm. The nucleus is usually larger than normal and frequently irregular in its configuration, the chromatin is not evenly distributed, nor are nucleoli present. The cytoplasm is more abundant than normal and comprises a large percentage of the cell area. It is frequently more basophilic than in the normal cell and may be cloudy and mottled in appearance rather than a clear, uniform hyaline blue. The cytoplasm is usually more granular than that of the normal lymphocyte, a condition which may be manifested by the presence of unusually large granules or of an unusually large number of small granules. Vacuolation of the cytoplasm or of the nucleus is common.

Although these cells are morphologically abnormal, they are mature and may appear in small numbers in almost any disease causing lymphoid hyperplasia. They are most numerous in infectious mononucleosis, of which they are the most characteristic feature. They must not be confused with the large immature lymphocytes encountered in lymphatic leukemia since the

PLASMA CELLS

There have been many theories advanced as to the origin and function of plasma cells, but no incontestable proof to support any one theory has been brought forward. Osgood believes they represent a separate and independent series of cells having no direct connection with lymphocytes, myelocytes, or monocytes, whereas most observers believe them to be a peculiar form of lymphocyte. They are present in small numbers in the bone marrow but appear in the peripheral blood stream infrequently. Only a few cases of plasma cell leukemia have been reported.

The cells are from 10 to 15 microns in diameter. Their most characteristic feature is to be found in the nucleus, which is round or oval and usually eccentrically placed in the cell. The chromatin tends to form in dense clumps that are most prominent at the periphery of the nucleus. The clumps are roughly triangular in shape in many instances, with their bases outward giving the nucleus a "clockface" or "cartwheel" appearance when the chromatin masses are sharply demarcated. The cytoplasm is a deep blue color, is frequently mottled, and occasionally contains a few reddish granules which do not give a peroxidase reaction. There is a perinuclear clear zone, and vacuoles in the cytoplasm are common.

TUERK CELL, TUERK IRRITATION CELL

The Tuerk cell is large, with an eccentric nucleus which stains a deep purple. The chromatin is commonly arranged in dense masses about the periphery leaving a central light staining area. The cytoplasm stains an intense deep blue and is frequently darker than the nucleus. It is usually mottled in appearance and often contains vacuoles. Such cells are rarely encountered in normal blood but are occasionally found with irritative lesions of the bone marrow or with diseases of the lymphoid tissues.

III MONOCYTES

The origin of the monocytes has been the subject of considerable discussion. Various authors have described them as arising from myeloblasts, lymphoblasts, monoblasts, endothelial cells, histiocytes, undifferentiated mesenchymal cells, or a combination of these sources. The present consensus among clinical hematologists identifies monocytes as a separate series of cells with an independent origin from a reticular cell of the reticulo-endo-

differential diagnosis between infectious mononucleosis and acute lymphatic leukemia rests primarily upon the structural characteristics of the cells.

Degenerated Cells

When a thin smear of the blood is made, a few cells will be broken up so that only the nucleus remains visible. This may occur with any type of cell but is particularly common with immature lymphocytes. In lymphatic leukemia, therefore, there may be a great many of these broken and degenerated cells, which are sometimes called "naked nuclei," "basket cells," or "smudges." The nucleus may be left intact even though the cell membrane has ruptured and the cytoplasm has disappeared. More frequently the nucleus is so spread out and thin that it is impossible to be sure to which type of cell it belonged. It may be smeared out in such a way as to form a large smudge, dense at one side but thinning out over a circular area with strands of chromatin radiating outward in a coarse interlacing network. This type has been called a "basket cell." Since such cells have been included in doing the total leukocyte count, they must be accounted for in the differential count and may be classified as basket cells, smudge cells, naked nuclei, or degenerated cells. They are particularly common in lymphatic leukemia, and since nucleoli are frequently seen in the nuclear material, it may be assumed that they were immature lymphocytes.

Function of Lymphocytes

Little is known of the function of the lymphocytes in the blood stream or of their life cycle. It is assumed that their span of life is short and that they are eliminated through the gastrointestinal tract, but this supposition is not well established. Lymphocytes are capable of some ameboid motion but are not as active as neutrophils. They aid in walling off chronic inflammatory lesions but they are not bactericidal or phagocytic. They may function by absorption and removal of toxins or, in the fixed lymphatic tissues, by production of antitoxins. It is of interest to note that many diseases which are followed by a lasting immunity are characterized by an increased lymphocyte count during the acute phase. Not all diseases characterized by a lymphocytic response produce an immunity, however. Lymphocytes are more numerous in certain infections such as measles, mumps, undulant fever, and whooping cough, as well as during the convalescent period of other acute infections. Their number may be increased with those diseases which cause an enlargement of the fixed lymphatic tissue and in infectious mononucleosis.

ers are visible through the overlying folds, giving an appearance of depth to the cell. This folding is not apparent in all cells, and the nucleus may be oval in shape, but there are usually one or more indentations in its outline (Fig. 8). The cytoplasm is blue in color but cloudy or mottled, and darker than the cytoplasm of the normal lymphocyte. The cytoplasm may be more densely stained at the periphery of the cell than close to the nucleus. There may be a few or many fine acidophilic granules within the cytoplasm. Cytoplasmic projections from the periphery of the cell are frequent, either as multiple small budlike processes or as larger rounded pseudopodium-like structures.

Mature Monocyte

The mature monocyte is the largest of the normal leukocytes in the blood stream. The nucleus is large and may be oval with a slight indentation or, more frequently, kidney or horseshoe shaped. It stains a rather deep reddish purple. The chromatin strands are coarse and dense and arranged in a loose meshed network. This network is evenly distributed with no clumping of the chromatin as in the lymphocyte, nor are there dense blocks such as are found in the granulocytes. The chromatin structure of the nucleus is one of the most distinctive features of the cell and should serve to differentiate it from other mature cells. The cytoplasm is a light blue, slightly cloudy and mottled, and is more densely stained at the periphery of the cell. There may be a particularly light staining area within the bend of the nucleus. A few or many fine acidophilic granules may be found in the cytoplasm. These are sometimes grouped together opposite the bend of the nucleus. They are much smaller and less distinct than the azure granules of the lymphocyte and give a slightly positive peroxidase reaction.

Functions of the Monocyte

Monocytes are phagocytic in the blood stream and may occasionally be found with engulfed erythrocytes or cell fragments. They are also phagocytic for bacteria and apparently migrate into areas of inflammation where they aid in the protective mechanism of the body. They are increased in number in monocytic leukemia and moderately so in certain chronic infections and in diseases accompanied by hypertrophy of the fixed lymphatic tissues, such as Hodgkin's disease and lymphosarcoma. A moderate increase in their number occurs during the stage of hematogenous spread in pulmonary tuberculosis and also in subacute bacterial endocarditis.

thelial tissues. From this cell, which is present in lymph nodes, spleen, bone marrow, and many other locations, is derived the monoblast or parent cell. It is not directly related to either the lymphocytic or the myelocytic series. True, the monoblast cannot be differentiated morphologically from the lymphoblast or myeloblast, but the absence of characteristic structural features in this immature cell is not sufficient evidence for placing the monocyte in the myeloid or lymphocytic series. It must be acknowledged that at the present time the origin of the monocytes has not been conclusively proved, but they probably represent a separate series which, in mature form, comprises about 4 or 5 per cent of the leukocytes of normal blood.

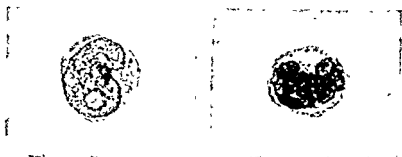


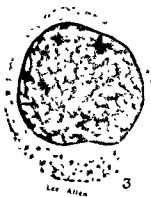
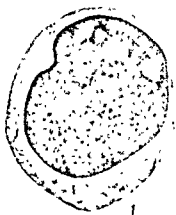
FIG. 8 Photomicrographs of monocytes. On the left is an immature monocyte with an irregular, folded nucleus showing nucleoli as found in a case of monocytic leukemia. On the right is a mature monocyte.

Monoblast

The monoblast is a large cell which from its structural appearance on the blood smear is indistinguishable from the blast cells of other series. The nucleus is large, has a fine chromatin network which stains a light purple with the polychrome dyes, and contains nucleoli. The cytoplasm is basophilic and may be somewhat darker and more mottled in appearance than that of the lymphoblast or myeloblast.

Immature Monocyte or Premonocyte

The intermediary stage in the development of the monocyte is a large cell averaging from 15 to 20 microns in diameter. It is characterized by a distinctive type of nucleus in which the rather dense chromatin strands stain a light purple color but form such a loose open network that the nucleus appears to be transparent. The nucleus is convoluted and folded so that it may be very irregular in shape. Because of its transparency, the deeper lay-



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SUPRAVITAL STAINING OF LEUKOCYTES

The descriptions given for the various types of cells have been based upon their appearance when stained on a fixed dry smear with a polychrome or Romanowsky type of stain (Wright's). The supravital method of staining, in which the cells are stained and examined in the living state, has certain advantages over fixed smear although it is impractical for routine clinical use. For this method of staining the glass slides are meticulously cleaned, washed, and dried, and the dye is applied to the slide in a very thin film and allowed to dry. A drop of fresh blood is placed on the prepared slide, a cover slip is applied and sealed with vaseline, and the examination is made on a warm stage microscope so as to preserve the life and motility of the cells. The dyes which are most commonly used are neutral red and Janus green.

With neutral red the neutrophilic granules of the granulocytic series stain a light pink, the eosinophilic granules a yellowish brown, and the basophilic granules a deep red. As the preparation is allowed to stand, vacuoles appear in the cytoplasm. These gradually increase in size and number. They apparently indicate metabolic activity and appear more quickly in the cells of patients having an acute infectious process. The adult cells of this series are highly refractile in appearance, and the lobulations in the nucleus are more apparent than on a fixed smear. They are actively motile. Mitochondria, which take the Janus green stain, are usually not demonstrable in the adult granulocytic cell, but are found in immature cells so that they will be apparent in the myeloblast although there is no reaction to the neutral red stain. In the myelocyte stage both mitochondria and red granules are present in the cell.

Monocytes present a variable appearance in so far as their size and shape are concerned. They may be rounded or oval, as on a fixed smear, they may be large and irregular with short blunt protruding processes, or they may be elongated. Neutral red vacuoles are numerous, and commonly from twelve to thirty vacuoles of varying sizes are present. These may be evenly dis-

PLATE V. THE MONOCYTIC SERIES

1. Monoblast. 2. Immature monocyte with irregular folded nucleus. 3, 4, 5. Monocytes with varying nuclear configuration

tributed throughout the cytoplasm or grouped together as a rosette opposite the bend in the nucleus. Vacuoles are not present in the protruding cytoplasmic processes. In addition to the red vacuoles there are mitochondria which have been stained by Janus green. These are small and greenish in color. They may be fairly evenly distributed throughout the cell and mixed with red vacuoles but more frequently they are encountered outside the area in which the vacuoles are found. The distribution of the red vacuoles in a rosette formation is characteristic of the monocyte. This arrangement, however, may be found in other types of cells as well. The monocyte is sluggish in its motility.

The lymphocytes are characterized by: (1) the small number of neutral red vacuoles which are present, frequently only two or four of these being found and seldom more than eight; (2) the numerous mitochondria, which are large and of uniform size, and (3) a clear cytoplasm. The red vacuoles may be found in any location in the cytoplasm with no characteristic distribution. The mitochondria tend to be clumped close to the nucleus, especially within the nuclear indentation. They are larger than those found in other types of cells, whereas the vacuoles are small and uniform in size and stain a deep red.

The supravital staining technic is of value as a supplementary measure to the ordinary fixed smear and as an aid in judging physiologic activity of the cells. It is not a procedure to supplant the fixed smear for routine differential counts.

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THE BLOOD PLATELETS OR THROMBOCYTES

THE BLOOD PLATELETS OR THROMBOCYTES ARE NOT, STRICTLY SPEAKING, cellular elements of the blood but the products of cells. They are not able to regenerate or reproduce themselves, and as they are destroyed in the performance of their functions, they must be replaced in the circulation. The normal life span of platelets in the blood stream is short, probably varying from a few hours to three or four days, so that these structures are replaced in the circulating blood at an approximate rate of 100,000 platelets per cubic millimeter of blood per day.

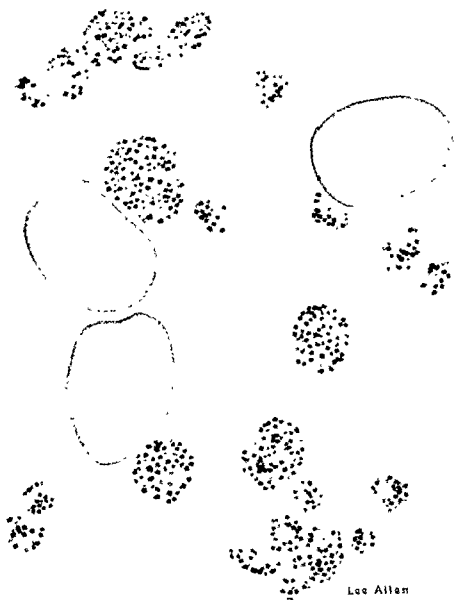
Origin of Platelets

Although many theories have been advanced to explain the origin of blood platelets, the predominating opinion at present is that they are produced from megakaryocytes and that the primary site of their production is the bone marrow. Megakaryocytes are extremely large cells each of which contains a relatively large multilobulated nucleus. The diameter of megakaryocytes is 35 to 40 microns, and the nucleus occupies the greater portion of each cell. The nucleus resembles that of a polymorphonuclear leukocyte in its configuration, but is larger, and the filaments connecting its lobules are coarser. The chromatin forms a coarse reticular network in the nucleus and stains a purplish color by Wright's method. In the cytoplasm of the cell is a peripheral clear zone which stains a light hyaline blue, but elsewhere the cytoplasm contains many fine purplish red granules. The cell is extremely variable in shape, sometimes it is round or oval and sometimes it presents a bizarre and irregular outline. Pseudopodia are frequently present. The origin of the megakaryocyte has not been definitely ascertained, but it probably arises from a primitive cell—the hemocytoblast—or from a fixed cell of the reticulo-endothelial tissue. Megakaryocytes are more numerous in the bone marrow than elsewhere in the body but also exist in the spleen, liver, and lungs in varying numbers. However, it is thought that those in

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the bone marrow are primarily the ones from which platelets are produced. Megakaryocytes do not get into the blood stream except under rare pathologic conditions.

Blood platelets are similar in their structure and staining reaction to the cytoplasm of megakaryocytes. They are probably broken-off particles of megakaryocytic cytoplasm which have been set free in the circulating blood. It was formerly believed that megakaryocytes were capable of ameboid motion and that their pseudopodia penetrated the lumen of blood vessels, subsequently breaking off within the vessels to become platelets. It is now thought that formation of platelets is independent of megakaryocytic pseudopodia and that it occurs when megakaryocytes begin to increase in size and their nuclei begin to divide. At this stage of development there is fragmentation or segmentation of megakaryocytic cytoplasm, and resulting particles of cytoplasm are carried away by the blood stream as platelets

Structure

Platelets are small, rounded, or irregular disk-shaped structures whose average diameter is 2 to 4 microns and whose average thickness is 0.5 to 1 micron. Their size and configuration are variable. Extremely large forms are frequently encountered under abnormal conditions. These may be round bodies equal in size to an erythrocyte or larger, or they may be rodlike structures whose length can be 25 to 30 microns. The average volume of normal platelets is approximately 10 to 12 cubic microns

Platelets may be found in many irregular and bizarre forms, particularly upon dry smears. Their staining reaction and structure can be varied greatly by different methods of fixation and staining. For this reason, too much significance should not be placed on their structural alterations. On the dry smear the platelet appears to consist of a peripheral clear zone with a clump of coarse granules in the center. In the living state the granular material is

PLATE VI.

A group of platelets with three erythrocytes for comparison as to size. These illustrate the variations in their size and shape as well as the internal granular structure of the intact platelet.

Thrombocytosis or Increased Platelet Count

There is a marked rise in the number of blood platelets after operations, which commonly reaches its peak on about the tenth postoperative day. The degree of increase roughly parallels the amount of tissue injury, being unusually high after bone operations and slight or absent following minor surgical procedures. The greatest increases follow splenectomy. Counts of 1,000,000 or more or values as high as 3 per cent by volumetric determination are frequent after removal of the spleen. Similar increases have been observed following trauma, especially after fractures of bone.

Platelets are more numerous in polycythemia vera, and the frequent occurrence of spontaneous intravascular thromboses in this disease is possibly due in part to thrombocytosis in addition to increased blood viscosity and sluggish blood flow. Thrombocytosis occurring after splenectomy and other operations may partially account for spontaneous thrombi and emboli which appear as postoperative complications of such procedures. Platelets occasionally increase in number in early chronic myelogenous leukemia, although late in the chronic form of the disease and in the acute forms a thrombopenia occurs. A permanent rise in number of platelets has been described as an idiopathic clinical entity. The increase after splenectomy may be permanent.

Thrombopenia or Lowered Platelet Count

Thrombopenic purpura provides the most pronounced example of a decrease in the number of platelets, and this may occur in either a primary or a secondary form. In a secondary form thrombopenic purpura can be found in association with many other diseases (Chapter XVI). The number of platelets is also moderately reduced in pernicious anemia and the related macrocytic anemias, Banti's syndrome, diseases of the liver, and as a terminal event in many blood dyscrasias. A mild grade of thrombopenia commonly occurs during the acute stages of infectious diseases, and can occasionally exist in association with many other physiologic and pathologic conditions.

Function of Platelets

Although it is universally agreed that platelets are intimately concerned with blood coagulation, their exact role in the clotting mechanism is disputed. Available evidence suggests that they play a dual role, first in accelerating coagulation of blood, second in promoting syneresis or clot retractility to form a firm and adherent clot.

By volumetric determination platelets represent 0.4 to 0.6 per cent of the total blood volume. Minor variations in numbers of platelets cannot be detected by volumetric determination, but variations great enough to be of clinical significance are definitely detectable by this technic. In certain



FIG. 9 Photomicrograph of a blood smear of platelets showing variations in the size and in the density of staining of the platelets. Although derived from a patient with thrombopenic purpura, these platelets are normal in appearance (Hal Downey, Handbook of Hematology Paul B Hoeber, Inc)

instances there can be a distinct drop in the platelet count, but it is possible for this drop to be compensated by large or giant forms of platelets. Under these circumstances volumetric determination is more precise than actual platelet counts because it indicates the amount of platelet material present regardless of individual size and number of platelets.

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There is abundant evidence to show that platelets influence blood coagulation but that they are not essential to the coagulative processes. Although platelets are rich in thromboplastin, there are other available sources of thromboplastic material, probably in blood plasma and tissue juice, so that coagulation will take place when platelets are absent. However, addition of platelets or of platelet extract accelerates coagulation of blood, and the substance liberated by destruction of platelets probably initiates blood coagulation under normal conditions. When this substance is absent, coagulation occurs, but onset of the process is delayed.

To accelerate or initiate blood coagulation platelets apparently increase the rate of conversion of prothrombin to thrombin. Platelets do not contain prothrombin, but upon disintegration thromboplastin is liberated. It is probable that an abundance of thromboplastin increases the rate of conversion of prothrombin to thrombin and thereby accelerates the rate of coagulation.

The second function of platelets in coagulation of blood is more easily demonstrated and more apparent in ordinary laboratory procedures than is the first. Clots produced by blood without platelets lack the firmness and rigidity of normal clots. They are soft, amorphous, jelly-like masses easily removed and easily broken and do not contract down to a firm mass or shrink away from the sides of the tube in which the blood has coagulated. When this type of nonadherent and nonretractile clot is formed at the site of an injury in vivo there can be leakage of blood about the periphery of the clot or the clot can be displaced from the damaged vessel. In either circumstance bleeding continues. When a normal clot is formed within the lumen of an injured vessel the edges of the vessel tend to be drawn together by the adherence and retraction of the clot. When platelets are absent the clot has little tendency to draw the edges of the vessel together, consequently bleeding is not adequately controlled. Bleeding time is therefore prolonged when platelets are few or absent even though coagulation time as ordinarily determined may be normal.

Platelets tend to agglutinate in large irregular masses or clumps, and also to adhere to foreign bodies. Some evidence indicates that they are involved in ridding the blood stream of foreign particles and bacteria and in immune and antibody reactions of the serum. Their exact role, if any, in these processes is unknown.

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globin, the amount of hemoglobin per unit of blood can be calculated. This method is used to standardize the simpler and more commonly used instruments, but it is not practical for clinical use.

For clinical determination the acid hematin method is most commonly used. A known amount of blood is diluted with tenth-normal hydrochloric acid to convert hemoglobin to acid hematin, which is brown in color. The intensity of the brown color is then compared to that of a fluid standard, a brown glass rod or prism, or a series of brown glass standards of varying intensities, and the amount of hemoglobin is computed from the depth of the color. In the Newcomer method a standard brown glass disk is inserted into one tube of a Duboscq colorimeter, and the unknown solution is compared to it. An advantage of acid hematin methods is that shades of brown are more easily matched by most observers than shades of red. A disadvantage is that the color of acid hematin gradually deepens for a period of about forty minutes, and readings must therefore be delayed for this length of time. Depth of color is also affected to some extent by nonhemoglobin substances of the blood plasma. Despite these disadvantages and individual inaccuracies in matching colors acid hematin methods are the most satisfactory of those available for routine clinical use. Instruments utilizing photoelectric cells for matching shades of brown in acid hematin mixtures are becoming more widely used. They are of great value and are precise in many respects, however, they do not eliminate all sources of error.

Other less often used methods utilize comparison of the color of diluted blood to red standards, but difficulty is frequently encountered in matching various shades of red. The Tallqvist scale, least precise of all commonly used procedures, requires matching the color of blood which has been collected on a piece of absorbent paper with shades of red on a standard representing 10 to 100 per cent hemoglobin. The carbon monoxide method of hemoglobin determination is precise but not practical for clinical use. A method by which the amount of hemoglobin is ascertained by determination of the iron content of blood not only is laborious but fails to take into account the nonhemoglobin iron of the blood, which is not an inconsiderable amount.

Normal hemoglobin levels reported by different observers vary, and it is difficult to set an absolute standard of normal. Osgood found 15.8 Gm. for men and 13.8 Gm. for women to be the average normal. Wintrobe, from a compilation of data, found in normal men values ranging from 14 to 18 Gm. with an average of 16 Gm., and in normal women values ranging from 12 to 16 Gm. with an average of 14 Gm. Our own results, which were obtained from determinations on young adults, have been slightly lower than the

NORMAL HEMATOLOGIC VALUES

TO INTERPRET RESULTS OBTAINED FROM A STUDY OF THE BLOOD IT IS NECESSARY to know not only normal values for its different constituents but also physiologic variations which can occur in the absence of any definite pathologic condition. Normal variations are great enough, in some instances, to cause confusion unless their significance is recognized. In other instances there are differences of opinion as to normal levels so that dividing lines between normal and abnormal are difficult to establish. The normal level of blood hemoglobin particularly is the subject of many divergent views. The present chapter is a brief recapitulation of normal values for various constituents of the blood to serve as a basis for interpretation of results obtained from hematologic studies on patients.

HEMOGLOBIN

Estimation of the amount of hemoglobin in a unit volume of blood is one of the most unsatisfactory of the common laboratory procedures. Values accepted as normal range from 13.8 Gm. to 17 Gm. per 100 cc. of blood. Because of this wide range it is not advisable to express the hemoglobin level in terms of percentage of normal unless at the same time the amount accepted as 100 per cent is specified. Obviously, to compare results expressed as percentages of normal but obtained by different methods it is necessary to transpose the percentage values into terms of grams per unit volume. Readings should therefore always be made and reported as grams of hemoglobin per hundred cubic centimeters of blood. There is no better reason for reporting hemoglobin levels on a percentage basis than for recording erythrocyte counts or blood pressures in percentage terms.

Several methods have been devised for determining the amount of hemoglobin in the blood. The most precise is the oxygen capacity method, in which the amount of oxygen which combines with a known amount of blood is ascertained. Since 1.34 cc. of oxygen combines with 1 Gm. of hemo-

is not associated with evidence of new cell formation and is probably the result of expulsion into the circulating blood of the reserve supply of erythrocytes. If a person remains in a region of low barometric pressure, evidence of increased erythrocytic production appears, and his bone marrow becomes hyperplastic. Anoxemia resulting from a low barometric pressure is a stimulus for erythropoiesis. Erythrocytes are more numerous as long as the individual remains at the high altitude, and the increase in number of cells is roughly proportional to the altitude.

Sex. Differences in hemoglobin and erythrocyte levels in males and females have been previously mentioned. These differences do not appear before puberty, and they become less marked in elderly subjects.

Muscular Activity. Exercise, especially strenuous exertion in a person of sedentary habits, is associated with an elevation in the erythrocyte count. This is a transient phenomenon and is undoubtedly due to a redistribution of existing cells.

Emotion. Excitement, fear, and other psychic reactions are followed by increased erythrocyte counts which are due in part to liberation of the stored erythrocytes in the spleen. Hemoconcentration with diminished plasma volume possibly plays a part in this increase of erythrocytes because erythrocyte counts have been observed to rise following psychic stimulation of splenectomized subjects.

Season. Erythrocyte counts tend to be slightly higher in summer than in winter. This difference is not great, and may be due to increased loss of fluids by perspiration in hot weather, resulting in hemoconcentration.

Water Balance. Intake of large amounts of fluids dilutes the blood sufficiently to lower the erythrocyte count to a slight degree. Dehydration from any cause produces sufficient hemoconcentration to elevate the count. Such dilution and concentration produce changes in numbers of cells per unit volume of blood rather than in actual numbers of cells in the circulation.

Diurnal Variation. Variations in numbers of erythrocytes and in hemoglobin levels are detected when determinations are made at regular intervals throughout the day and night. Such variations may amount to 10 per cent. They are probably results of activity, emotional factors, fluid intake, and inherent errors in methods of determination.

Erythrocyte Diameter

The diameter of erythrocytes can be measured directly on dried blood smears by use of a micrometer disk in the eyepiece of a microscope, or the mean diameter can be ascertained by use of a suitable instrument (halometer,

above values, and similarly low values have been noted by other observers in this region. Doan has stated that each healthy adult establishes an equilibrium for red cells and hemoglobin at the optimum for his oxidation requirements, but has an available reserve supply of red cells in the splenic pulp. Thus consecutive counts can show considerable variation in the number of erythrocytes, and fluctuations of 1 to 2 Gm. of hemoglobin can occur within a short period of time. Results of studies carried out in our laboratory are in agreement with Doan's conclusions. It seems also that there are great individual variations in normal hemoglobin levels so that a reading which is normal for one individual may represent a mild anemia in another.

Physiologic variations which affect the hemoglobin level are the same as those affecting the erythrocyte count and will be discussed more fully under that heading. Diurnal variations, increases with muscular activity or with psychic factors such as excitement or fear, and increases at high altitudes have been observed. Hemoglobin levels are high at birth but fall rapidly during the first weeks of life, and we have found a slight decrease in hemoglobin in elderly subjects. Hemoglobin values are somewhat higher in summer than in winter, but no apparent difference has been noted between the values for inhabitants of tropical and nontropical countries.

ERYTHROCYTES

Total Erythrocyte Count

Normal values for erythrocyte counts are usually considered to be 5,000,000 per cubic millimeter for males and 4,500,000 for females. Osgood found these levels slightly low. His counts averaged 5,400,000 and 4,800,000 for males and females respectively.

Physiologic Variations

Age. At birth hemoglobin levels and erythrocyte counts are high; however, they begin to fall after the first few days of life and reach normal levels after a few weeks. These changes are discussed more fully in Chapter XXII. Studies which were carried out on a group of elderly subjects show that a slight decrease in hemoglobin levels and erythrocyte counts is encountered in older patients. The average hemoglobin level for 100 elderly subjects was 13 Gm. per 100 cc. of blood, and erythrocyte counts averaged 4,580,000. The difference between values for males and females was less pronounced than in younger individuals.

Altitude. A rapid increase in the number of erythrocytes and amount of hemoglobin appears during an ascent from low to high altitude. This change

is not associated with evidence of new cell formation and is probably the result of expulsion into the circulating blood of the reserve supply of erythrocytes. If a person remains in a region of low barometric pressure, evidence of increased erythrocytic production appears, and his bone marrow becomes hyperplastic. Anoxemia resulting from a low barometric pressure is a stimulus for erythropoiesis. Erythrocytes are more numerous as long as the individual remains at the high altitude, and the increase in number of cells is roughly proportional to the altitude.

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Altitude. A rapid increase in the number of erythrocytes and amount of hemoglobin appears during an ascent from low to high altitude. This change

Example:

Actual reading = 11 Gm.

$$11 \times 6.9 = 76\%$$

For convenience in expressing certain relationships between number, size, and hemoglobin content of erythrocytes certain so-called indices are commonly used. These are an aid in study and classification of various types of anemia although they are less precise than expression of erythrocyte size and hemoglobin content of the erythrocyte in absolute values.

Color Index

The color index is the ratio of hemoglobin level expressed as percentage of normal to red cell count expressed as percentage of normal, and indicates the amount of hemoglobin per red cell in relation to normal. In a normal individual both hemoglobin level and erythrocyte count are 100 per cent of normal, therefore the color index is $1.0 \left(\frac{100\%}{100\%} \right)$. A positive color index, greater than 1.0, indicates that each erythrocyte contains more than the normal amount of hemoglobin, and a negative color index, less than 1.0, indicates a lowered hemoglobin content per cell.

$$\text{Color index} = \frac{\text{Hemoglobin—\% of normal}}{\text{Erythrocytes—\% of normal}}$$

Volume Index

The volume index is the ratio of volume of packed erythrocytes to the number of erythrocytes per unit volume of blood, and indicates the relative size of erythrocytes compared to normal. A volume index greater than 1.0 indicates the average size of the individual erythrocyte to be greater than normal.

$$\text{Volume index} = \frac{\text{Hematocrit—\% of normal}}{\text{Red cell count—\% of normal}}$$

Saturation Index

The saturation index is the ratio of amount of hemoglobin to volume of packed erythrocytes, and indicates concentration of hemoglobin in the red cell mass rather than in individual cells.

$$\text{Saturation index} = \frac{\text{Hemoglobin—\% of normal}}{\text{Hematocrit—\% of normal}}$$

These indices have certain inherent disadvantages, chief among which is the unpreciseness of percentages based on normal values which are not ac-

erimeter, erythrocytometer) in which diffraction of a beam of light passing through the dried smear is observed by projection on a suitable screen or disk. The average normal diameter is 7.2 to 7.5 microns.

Hematocrit

The term *hematocrit* means the volume of erythrocytes packed by centrifugation in a given volume of blood. Hematocrit is expressed as the percentage of the total blood volume which is composed of erythrocytes, or as volume in cubic centimeters of centrifugally packed erythrocytes in 100 cc. of blood. Average normal values are about 45 for males and 42 for females.

Computation of Indices and Coefficients

For determination of various indices and other calculations it is necessary to accept certain values of hemoglobin, number of red cells, and hematocrit as normal and to express observed values in terms of percentages of normal. For this purpose Wintrobe has used 5,000,000 cells as the equivalent of 100 per cent of normal for the erythrocyte count, and has accepted 14.5 Gm. as the *hemoglobin coefficient*—the normal hemoglobin value which corresponds to an erythrocyte count of 5,000,000. The normal hematocrit value which corresponds to 5,000,000 erythrocytes has been set at 43.2 cc. per 100 cc. of blood. To calculate quickly percentages of normal for various determinations the following methods can be used:

Erythrocytes: $5,000,000 = 100\%$

$$\text{Actual count in millions} \times 20 \left(\frac{100}{50} \right) = \text{per cent of normal}$$

Example:

$$\begin{aligned} \text{Actual count} &= 3,500,000 \\ 35 \times 20 &= 70\% \end{aligned}$$

Hematocrit: $43.2 = 100\%$

$$\text{Volume per 100 cc. of blood} \times 2.3 \left(\frac{100}{43.2} \right) = \text{per cent of normal}$$

Example:

$$\begin{aligned} \text{Actual reading} &= 35 \\ 35 \times 2.3 &= 80\% \end{aligned}$$

Hemoglobin: $14.5 = 100\%$

$$\text{Grams per 100 cc.} \times 6.9 \left(\frac{100}{14.5} \right) = \text{per cent of normal}$$

10,000 are usually considered as indicative of leukocytosis, and counts of less than 5000 as indicative of leukopenia. Certain normal individuals, however, have leukocyte values somewhat above or below these levels.

Differential Count

Myeloid Series

Neutrophils. Neutrophils are more numerous than any other type of leukocyte in the blood stream and represent 55 to 70 per cent of all white cells in the circulating blood. They normally occur in the blood stream as (1) band or nonsegmented forms, in which there is no separation of the nucleus into lobules, and (2) segmented or filamented forms, in which the nucleus is separated into two or more lobes connected by strands of chromatin

IMMATURE			YOUNG	ADULT			
MYELOBLAST	MYELOCYTE	METAMYELOCYTE	BAND	SEGMENTED	SEGMENTED	SEGMENTED	SEGMENTED
ONE LOBE	ONE LOBE	ONE LOBE	ONE LOBE	TWO LOBE	THREE LOBE	FOUR LOBE	FIVE LOBE
NON-FILAMENTED	NON-FILAMENTED	NON-FILAMENTED	NON-FILAMENTED	FILAMENTED	FILAMENTED	FILAMENTED	FILAMENTED

FIG. 10. Diagrammatic sketch of the stages in the maturation of a neutrophil. This shows the basis for the Schilling, Arnetz, and segment-nonsegment differential counts.

material (Fig. 10). Separate enumeration of band and segmented forms is known as a Schilling count, and information thus obtained is so valuable that a Schilling count should be performed as a part of every blood examination.

Band neutrophils represent 3 to 5 per cent of total leukocytes in the blood and are the youngest forms of the myeloid series of cells in the blood stream under normal conditions.

Segmented neutrophils comprise 50 to 65 per cent of leukocytes. A majority of the segmented forms have two or three lobes in the nucleus, but five or more nuclear lobes are occasionally present. When neutrophils are classified according to the number of segments in their nuclei the distribution is normally as follows:

Band or nonsegmented	5%
2 lobes	35%
3 lobes	41%
4 lobes	17%
5 lobes	2%

curately established. It is therefore more satisfactory to express findings as absolute values rather than in terms of percentage of normal. Mean corpuscular hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin concentration give in absolute terms information expressed in color index, volume index, and saturation index respectively. This information in absolute values is more reliable and more precise than are the various indices.

Mean corpuscular hemoglobin:

$$\text{M. C. H.} = \frac{\text{Hemoglobin grams per 1000 cc. of blood}}{\text{Red cell count, millions per cu. mm. of blood}}$$

Average normal value $\approx 29 \pm 2$ micromicrograms

Mean corpuscular volume:

$$\text{M. C. V.} = \frac{\text{Volume of packed erythrocytes per 1000 cc. of blood}}{\text{Red cell count, millions per cu. mm. of blood}}$$

Average normal value $\approx 87 \pm 5$ cubic microns

Mean corpuscular hemoglobin concentration:

$$\text{M. C. H. C.} = \frac{\text{Hemoglobin grams per 100 cc. of blood} \times 100}{\text{Volume packed red cells per 100 cc. of blood}}$$

Average normal value $\approx 34\% \pm 2$

The average total blood volume is 65 to 80 cc. per kilogram of body weight, or 6 to 9 per cent of total body weight. The average specific gravity of blood is about 1.052, and the viscosity is 4.5 compared to distilled water.

Erythrocytes are remarkably constant on blood smears in their size, shape, and staining reaction, and use of vital stains indicates that 0.2 to 0.8 per cent of erythrocytes are reticulated.

LEUKOCYTES

Total Leukocyte Count

Total leukocyte counts performed on normal adults result in values of 5000 to 10,000 white cells per cubic millimeter of blood with an average value of about 7000. When an individual is at rest under basal conditions, white cell counts give values of 5000 to 7000. Activity, among other things, increases the total number of leukocytes in circulating blood so that counts can vary considerably from hour to hour and from day to day when performed on the same normal person. Total leukocyte counts of more than

10,000 are usually considered as indicative of leukocytosis, and counts of less than 5000 as indicative of leukopenia. Certain normal individuals, however, have leukocyte values somewhat above or below these levels.

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Band or nonsegmented	5%
2 lobes	35%
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4 lobes	17%
5 lobes	2%

Eosinophils. Eosinophils represent 2 to 4 per cent of the leukocytes and seldom contain more than two lobes in their nuclei. They are fragile cells and are frequently found on stained smears with cell membranes broken and granules scattered about in the regions of the nuclei.

Basophils. Basophils are the least numerous of myeloid cells and often are not encountered on routine differential counts. They seldom comprise more than 1 or 2 per cent of leukocytes.

Lymphocytes

Lymphocytes make up 25 to 35 per cent of the white blood cells. A majority of lymphocytes in the blood stream are the small mature type with a narrow rim of cytoplasm, however, a few of the larger forms having more abundant cytoplasm can be observed. About 30 per cent of normal lymphocytes contain azure granules.

Monocytes

Monocytes, largest of normal blood cells, comprise 5 to 10 per cent of leukocytes. A coarse network of nuclear chromatin, and fine eosinophilic granulations in basophilic cytoplasm distinguish these cells from large lymphocytes. Most monocytes have horseshoe-shaped nuclei, but some nuclei are oval or irregular in shape.

Physiologic Leukocytosis

Numerous factors can cause the total leukocyte count to increase without any demonstrable pathologic condition. In newborn infants counts are high, frequently 20,000 or more, but subside to lower levels within a few days, although relatively high counts prevail during childhood.

Exercise increases the total number of leukocytes, but similar increases occur during fright, anger, and emotional stimuli so that it is not certain whether this response is due to muscular activity itself or to increased epinephrine secretion. Since the leukocytic response to exercise occurs in splenectomized animals, it cannot be due entirely to contraction of the spleen. The leukocytosis occurs rapidly, and immature cells do not increase in percentage, so that it seems to be due to redistribution of existing cells rather than to increased production of cells by hematopoietic centers.

A similar leukocytosis has been noted after convulsive seizures, attacks of paroxysmal tachycardia, and pain.

Studies of the influence of temperature on total leukocytes are somewhat at variance as leukocytoses have occurred after cold baths as well as after

hyperpyrexia caused by artificial fever therapy. Slight leukocytoses occur while individuals are becoming accustomed to high altitudes. They can also be observed during the third trimester of pregnancy, the average count being about 15,000. Total counts can increase during labor, but they become normal after four or five days.

Sabin has demonstrated a rhythmic diurnal variation in total numbers of leukocytes so that counts vary in the same individual from day to day and from time to time during the day. Highest peaks occur late in the morning and late in the evening. It appears probable that this rhythmic delivery of cells to the circulation accounts for the variations formerly ascribed to the effect of food, and the idea of "digestive leukocytosis" has been discarded.

The mechanism whereby leukocyte counts are maintained within relatively constant limits has not been ascertained, nor has any substance which definitely influences maturation of leukocytes been discovered. Nucleic acid, possibly derived from destroyed leukocytes, may have some influence on leukocyte production. Probably most physiologic leukocytoses are results of redistribution of existing cells and changes in blood volume due to altered water balance, because increases in total numbers of cells appear rapidly and in most instances differential counts remain unaltered without increases in percentages of band neutrophils to indicate that young cells are being thrown into the circulation. An exception is the leukocytosis of the newborn.

Physiologic leukocytoses in themselves are not important, but it is essential to keep them in mind in evaluating leukocyte counts so as not to interpret them as evidence of infection or other pathologic condition. Diurnal variations in counts must also be kept in mind when evaluating series of repeated leukocyte counts, as too much emphasis is not infrequently placed on relatively minor changes in a series of counts.

PLATELETS

The total number of blood platelets normally ranges from 200,000 to 400,000 per cubic millimeter of blood, but higher values have been reported by some observers. When measured volumetrically, platelets represent from 0.4 to 0.6 per cent of total blood volume. On blood smears they appear as small round or oval bodies occurring singly or in variously sized groups in which they can be so clumped together as to prevent careful morphologic study. Most platelets are small, but occasional giant forms exist which are as large as erythrocytes. In small platelets granular material is grouped together in a dense mass in the center, whereas in larger platelets the gran-

ular material is diffusely distributed throughout the cytoplasm. Only rough approximations of numbers of platelets can be made from examinations of smears. Causes for increased and decreased numbers of platelets have been discussed in Chapter IV.

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LEUKOCYTOSIS AND LEUKOPENIA

THE NUMBER OF LEUKOCYTES IN THE PERIPHERAL BLOOD STREAM OF A NORMAL adult ranges from 5000 to 10,000 cells per cubic millimeter of blood, averaging about 7000. A total leukocyte count above 10,000 is termed a leukocytosis. The leukocytosis which occurs as a result of infection or other abnormal stimulus disturbs the normal ratio between the various types of cells, *the increase in the total count being due primarily to an increase in the number of one particular type of leukocyte.* Neutrophils are the cells most frequently increased in number, and when this is the case, the condition is termed a leukocytosis or neutrophilia. Eosinophilia and basophilia represent leukocytoses in which the eosinophils or basophils are predominantly affected. Lymphocytosis and monocytosis signify an actual increase in the number of lymphocytes or monocytes

LEUKOCYTOSIS

Neutrophilic Leukocytosis, Neutrophilia

Causes

Infection. The most common cause for neutrophilia is infection. The height of the leukocyte count is, in general, in direct proportion to the acuteness and severity of the infection, but the leukocytic reaction is modified by the type of invading organism and by the resistance of the body. Infections with pyogenic or pus-forming organisms cause a higher leukocyte count than do other organisms, the response being greater with infections due to *the staphylococcus, streptococcus, pneumococcus, and meningococcus.* A higher leukocyte count usually results with localization of the infection and abscess formation than with the same organism circulating in the blood stream without a point of localization. This suggests that destruction of tissue is one factor in calling forth a leukocytic response and that bacteria and their toxins are not the only stimulating mechanisms. A very high leukocyte

count is common with pneumococcic pneumonia, meningococcic meningitis, and abscess, mastoiditis, or peritonitis due to pyogenic organisms. The height to which the white count will rise is affected by many conditions, but the response in a debilitated or cachectic patient is usually less than in one who is otherwise healthy. A low leukocyte response in the presence of a pyogenic infection may indicate that the infection is mild, but if there is an acute and extensive infection, the poor leukocyte response indicates low resistance and a poor prognosis. An excessively high leukocyte response is also of grave significance as it suggests that a last effort is being made by the defense mechanism of the body to cope with an overwhelming infection. The response in children is usually greater than that encountered in an adult with a similar type of infection, but even in adults a count of 30,000 or 40,000 is not uncommon in severe infections, particularly in pneumonia.

A leukocytosis is due to the formation and liberation of additional cells by the bone marrow. The marrow ordinarily releases only mature cells, and a majority of the neutrophils in normal blood have segmented nuclei. Only about 4 or 5 per cent are young forms with band or nonfilamented nuclei. Under the demands of an infection, however, there is an increased rate of production; consequently, more immature cells are liberated into the blood stream. This results in an increased percentage of band or nonsegmented neutrophils in the peripheral blood. This "shift to the left" is an indication of bone marrow stimulation rather than of infection itself. The rate at which these cells can be formed is surprisingly high as is shown by the rapid increase in the leukocyte count when it is followed at hourly intervals in patients with a severe infection.

Evidences of degeneration may be found in the neutrophils in severe infections. There may be toxic granulation, the cytoplasmic granules becoming larger, more deeply stained, and more prominent than normal. Vacuolation of the cytoplasm or of the nucleus is another sign of degeneration of the cell, and there may be one or many small unstained vacuoles. Extensive toxic granulation and vacuolation suggest that the patient's resistance is poor and point toward a less favorable prognosis. By determining the percentage of cells showing these changes the so-called "degenerative index" is found.

Posthemorrhagic. A neutrophilic leukocytosis follows acute hemorrhage if the loss of blood is extensive. The leukocyte count under these circumstances may reach 20,000 or 30,000, and the accompanying "shift to the left" indicates bone marrow stimulation. The count begins to rise twenty-four

to forty-eight hours after the hemorrhage, reaches its peak within a few days, and gradually subsides to the normal level.

Postoperative. A postoperative leukocytosis is encountered after extensive surgical procedures, probably resulting from a combination of tissue damage and loss of blood. It persists but a few days and then subsides. It must not be considered an evidence of postoperative infection.

Coronary Occlusion. The leukocytosis which follows occlusion of a coronary artery is usually moderate in degree, but the count may reach 12,000 or 15,000. There is a nuclear shift to the left indicating that the higher count is due to an increased production of cells rather than to a simple redistribution. This gives conclusive evidence that tissue damage is effective in producing a leukocytosis even in the absence of infection.

A sterile abscess or the intramuscular injection of inert material may also cause a leukocytosis.

Malignant Growths. Some malignant growths may be accompanied by a leukocytosis, particularly those in a far advanced stage, but this is not a constant finding. Secondary infection does not account for the reaction.

Intoxications. Intoxication of various types may be accompanied by a mild leukocytosis. This may occur with uremia, diabetic acidosis, and various drugs and chemicals.

Eosinophilic Leukocytosis

The eosinophils normally comprise 2 to 4 per cent of the white blood cells. They tend to disappear from the blood stream during an acute infection but may return in increased numbers during the convalescent stage. Little or no significance should be placed on an eosinophilia until it is above 8 or 10 per cent.

Causes

Allergy Allergic conditions such as hay fever, bronchial asthma, angio-neurotic edema, urticaria, eczema, and allergic rhinitis are commonly accompanied by a moderate increase in the number of eosinophils. Certain gastrointestinal diseases and some forms of arthritis are apparently on an allergic basis and may be accompanied by such a reaction. The eosinophilia which is occasionally found in tuberculosis, scarlet fever, and rheumatic fever is probably due to an allergic reaction to the bacteria or their toxins. A few cases of familial eosinophilia have been reported and are probably due to a familial allergic tendency.

Parasitic Infestations. Trichiniasis produces a marked eosinophilia, and

other parasitic infestations frequently evoke a similar response, but the absence of an increase in the eosinophils does not exclude the possibility of parasitic disease. An eosinophilia is sometimes encountered with roundworms (*Ascarides lumbricoides*), tapeworms (*Cestodas*), hookworms (*Ancylostomas*), pinworms (*Enterobius vermicularis*), filariasis, and *Echinococcus* infestation. The eosinophils may comprise 80 or 90 per cent of the leukocytes in these parasitic diseases, but the usual response ranges from 10 to 30 per cent.

Skin Diseases. Eosinophilia may accompany certain diseases of the skin but is not a constant feature of dermatoses. Identical skin lesions may produce an eosinophilia in one patient but not in another. Lesions of the skin which cause destruction of epithelial cells are the ones commonly associated with an increased eosinophil count. Scarlet fever is the only acute exanthem in which eosinophilia occurs.

Myelogenous leukemia. Myelogenous leukemia produces an increase in the number of eosinophils, but the height of the eosinophil count is variable.

Hodgkin's Disease. The presence of eosinophils in the involved lymph nodes is one of the distinctive and constant histologic features of Hodgkin's disease. In a few cases of the disease there is a marked eosinophilia in the peripheral blood, but this is far from common; a majority of patients have no increase in their eosinophil count. The incidence of eosinophilia in Hodgkin's disease is frequent enough, however, so that the disease must be considered in the differential diagnosis in all patients having an unexplained eosinophilia. We have observed one patient with an eosinophilia of 85 per cent. Carcinoma of the uterus and a few other malignant growths are occasionally accompanied by eosinophilia.

Miscellaneous Causes. Splenectomy is regularly followed by leukocytosis, and in some instances there is an increase in the number of eosinophils.

An eosinophilia was commonly encountered in patients with pernicious anemia whose therapy consisted of whole raw liver. It is seldom if ever encountered when liver extract is given parenterally.

Periarteritis nodosa is accompanied by varying degrees of eosinophilia, occasionally reaching as high as 50 per cent.

Tropical eosinophilia has been reported as a disease entity in which there are fever, malaise, cough, and evidences of bronchitis on examination and X-ray. The eosinophilia is marked. It has been suggested that a parasitic infestation is probably the basic factor in such cases.

Loeffler's syndrome consists of a transitory lung infiltration of short dura-

tion giving signs indicative of early pneumonia. It is sometimes associated with roundworm infestations although this etiologic factor has not been found in any of the cases under our observation. It is presumably allergic in origin and is accompanied by an eosinophilia.

Basophilic Leukocytosis, Basophilia

The basophils normally comprise from 0.5 to 1 per cent of the leukocytes. They are seldom significantly increased in number. A larger number of basophils may be found in myelogenous leukemia, but their ratio to the other granulocytes is seldom increased. They are occasionally more numerous in polycythemia vera and in some cases of cirrhosis of the liver.

LYMPHOCYTOSIS

Lymphocytosis is an increase in the number of lymphocytes per unit volume of blood. It is essential to distinguish between true lymphocytosis, in which there is an actual increase in the number of lymphocytes, and a relative lymphocytosis, in which the percentage of lymphocytes is increased without an actual increase in number. A relative lymphocytosis occurs when the total leukocyte count and the number of granulocytes are decreased while the lymphocytes remain constant.

The increase in number of lymphocytes which occurs with lymphocytic leukemia and infectious mononucleosis will be discussed in the chapters devoted to these diseases, and the lymphocytosis of infants and children is considered in Chapter XXII, "Hematology in Infancy and Childhood."

The number of lymphocytes is seldom increased in acute infectious diseases except in whooping cough. A true lymphocytosis is a characteristic feature of this disease and may be so striking that it suggests leukemia. A lymphocytosis occasionally occurs in active tuberculosis, mumps, measles, and undulant fever but is not a constant feature in any of these diseases. Lymphocytosis is a common finding during the convalescent stage after an acute infection even though lymphocytes do not increase in number during the acute stage.

The lymphocytes are frequently but not consistently more numerous in diseases characterized by enlargement of fixed lymphatic tissues, such as the early stage of Hodgkin's disease, lymphosarcoma, and infectious lymphadenopathy.

Acute infectious lymphocytosis is an infectious disease characterized by fever, vomiting, and abdominal distress, and a marked lymphocytosis which

persists after the other manifestations of the disease have subsided (see page 402).

A relative lymphocytosis is far more common than an absolute increase, being encountered in all cases in which there is a decrease in the number of neutrophils.

MONOCYTOSIS

Monocytes comprise from 5 to 10 per cent of the leukocytes under normal conditions, the average value being around 5 per cent. Monocytosis means an increase in the number of these cells above their normal value, but such a reaction is not common and seldom reaches a high level.

Monocytes play an important role in the formation of tubercles in pulmonary tuberculosis and by their phagocytic action aid in combating this infection. They may be more numerous in the circulating blood when the pulmonary lesion is active, but rather than being a favorable sign, this indicates activity and spreading infection. The ratio of monocytes to lymphocytes in the blood stream is normally about 1:3 (M:L ratio). When this ratio is altered during pulmonary tuberculosis so as to increase the number of monocytes in relation to the lymphocytes, it is an unfavorable prognostic sign.

In a few other bacterial infections there may be a moderate monocytosis. This occurs occasionally, but not consistently, in undulant fever, subacute bacterial endocarditis, and typhoid fever. In addition to these bacterial infections the monocytes are increased in number in other types of infection such as malaria, kala-azar, and Rocky Mountain spotted fever. There may be a temporary rise in the monocyte count during the convalescent period following acute infections.

Monocytosis may occur in those diseases in which there is a generalized lymphadenopathy such as Hodgkin's disease. In infectious mononucleosis the lymphocytes are predominantly affected, but there is also a moderate degree of monocytosis although it is overshadowed by the lymphocytic response.

LEUKEMOID REACTIONS

Leukemoid reaction is the term applied to those conditions in which there is a leukemic-like reaction of the white blood cells as a result of an infection or other stimulus. Such a reaction may be granulocytic, lymphocytic, or monocytic depending upon the type of cell involved. The reaction may

be manifested by (1) an exceptionally high total leukocyte count with the appearance of a few or many immature cells or (2) a great many immature cells without a great increase in the total leukocyte count. The first type of reaction is the most frequent. A leukemoid reaction is more common in children than in adults and is more frequently due to an infection than to other forms of marrow stimulation.

Infections occasionally produce a hyperleukocytosis in which the white cell count reaches 100,000 or more with a corresponding increase in the percentage of band neutrophils and a few metamyelocytes or myelocytes in the blood stream. We have observed such a reaction in a splenectomized patient with pneumonia, in whom nucleated erythrocytes as well as many immature leukocytes appeared in the blood stream. Exceptionally high leukocyte counts have been recorded with meningitis, pneumonia, pneumococcal endocarditis, empyema, smallpox, chickenpox, septicemia, miliary tuberculosis, and other diseases. The reaction may occur with almost any type of infection.

A leukemoid blood picture may occur with malignant growths which have metastasized to bone. Such metastases are prone to cause stimulation or irritation of the marrow, and immature myeloid cells are released into the blood stream. The total white count is usually not particularly high under these circumstances, but the anemia and immaturity of the leukocytes suggest leukemia. A similar response has been encountered with multiple myeloma.

Leukocytosis follows a severe hemorrhage and an exaggerated response may occur with immature cells appearing in the blood stream. Severe burns, mustard gas poisoning, and mercury poisoning have likewise produced this type of reaction. Heck mentions the recovery stage of pernicious anemia as occasionally showing a leukemoid reaction with excessive leukocytosis and myelocytes in the peripheral blood stream. The hemolytic anemias, particularly during infancy, are apt to have an exceptionally high leukocyte count with many immature cells.

Lymphocytic and monocytic reactions may also simulate their respective types of leukemia. A monocytic response of this magnitude is not common but has been observed in patients with severe infection of the gums and mouth. A leukemoid reaction of the lymphocytic type is frequent with infectious mononucleosis. Krumbhaar has observed immature lymphoid cells in the blood stream as a result of infections, and extremely high lymphocyte counts have been encountered in whooping cough, chickenpox, and osteomyelitis.

persists after the other manifestations of the disease have subsided (see page 402).

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also present. The principal offending substances are discussed under aplastic anemia (Chapter X) and agranulocytosis (Chapter XIX). Chemicals and drugs containing a benzene ring in their chemical structure are the chief offenders but gold salts, arsenic, dinitrophenol, sulfonamides, barbiturates, thiouracil, bismuth, and quinine have been incriminated. Leukopenia is one of the common complications and contraindications for the continued use of the sulfonamides and thiouracil.

Irradiation by radium, roentgen rays, and radioactive chemicals commonly depress the bone marrow although a selective effect on the leukocytes may occur from relatively slight exposure.

The nitrogen mustards regularly produce a leukopenia and this is one of the complications which regulates the frequency of their administration.

Leukopenia may be found in cachectic and debilitated patients and has been noted in certain nutritional deficiencies such as sprue and pellagra.

Various blood dyscrasias present leukopenia as a part of their hematologic picture. In both pernicious anemia and aplastic anemia it is an integral part of the disease. It is also found in aleukemic leukemia of the myelogenous, lymphocytic, or monocytic type and in some myelophthisic anemias in which the hematopoietic bone marrow is crowded out by other tissues.

Banti's syndrome and Felty's syndrome have leukopenia as one of their characteristic features, and in Hodgkin's disease and lymphosarcoma it is occasionally present. Primary splenic neutropenia and panhematopenia are discussed elsewhere (Chapter XIX).

Diseases of the liver such as catarrhal jaundice and portal cirrhosis are commonly accompanied by leukopenia.

Myxedema presents a mild leukopenia in some instances, as do Gaucher's disease and lupus erythematosus disseminatus.

Agranulocytosis is a specific disease entity in which there is a marked leukopenia of sudden onset with almost complete absence of neutrophils from the blood stream. It is discussed separately in Chapter XIX.

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LEUKOPENIA

Leukopenia, a reduction in the total leukocyte count below 5000, occurs in a wide variety of conditions. The lowered count in practically all of these conditions is due to a reduction in the number of neutrophils, which causes a neutropenia and a relative lymphocytosis. The reduction in the number of cells may be brought about by: (1) a decreased rate of production or liberation of the cells, (2) increased destruction of the cells, or (3) redistribution of the cells within the vascular bed.

A decrease in the production of cells is the most common cause of leukopenia and is apparently the reason for the leukopenia which is encountered in most types of infection, whether it be bacterial, virus, or protozoal in origin. It also accounts for the leukopenia encountered in those conditions in which the bone marrow is destroyed or replaced as in the myelophthisic anemias.

The question of a splenic hormone which regulates the activity of the bone marrow has not been settled so that it is impossible to say whether or not such a substance exists. There are instances, however, such as in primary splenic neutropenia and panhematopenia, in which a leukopenia develops either as a result of excessive destruction of the cells by an overactive spleen or because of a disturbance of the regulatory mechanism of the spleen.

A redistribution of the cells in the vascular bed may account for the leukopenia encountered in anaphylactoid shock and foreign protein reaction.

The bacterial infections which commonly produce a leukopenia are typhoid, paratyphoid, and undulant fever, but it is occasionally encountered with subacute bacterial endocarditis and infectious or atrophic arthritis. Severe infections with other organisms, such as a pneumococcic infection or miliary tuberculosis, will occasionally cause a leukopenia.

Virus infections which may cause leukopenia include influenza, measles, rubella, psittacosis, dengue, and German measles. Infectious mononucleosis may also show a leukopenia early in the course of the disease, and the same is true of smallpox.

Protozoal infestations in which leukopenia may be a feature are malaria, relapsing fever, and kala-azar.

The leukopenia associated with these infections is usually mild, seldom reaching extremely low levels, so that leukocyte counts of from 3000 to 4000 are most frequently encountered. The cause of the leukopenia is probably a toxic inhibition of the leukopoietic bone marrow.

Certain chemicals and drugs may cause leukopenia although most of these result in a depression of all the hematopoietic elements of the bone marrow rather than causing only a leukopenia, so that anemia and thrombopenia are

also present. The principal offending substances are discussed under aplastic anemia (Chapter X) and agranulocytosis (Chapter XIX). Chemicals and drugs containing a benzene ring in their chemical structure are the chief offenders but gold salts, arsenic, dinitrophenol, sulfonamides, barbiturates, thiouracil, bismuth, and quinine have been incriminated. Leukopenia is one of the common complications and contraindications for the continued use of the sulfonamides and thiouracil.

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CLASSIFICATION AND GENERAL DISCUSSION OF ANEMIA

2. ANEMIA DENOTES A REDUCTION IN THE NUMBER OF ERYTHROCYTES OR THE amount of hemoglobin per unit volume of blood. It may result from a great many causes and is accompanied by various changes in the appearance of the erythrocytes. The common classifications of anemia are based either on the structural changes in the erythrocytes or on the etiology and pathogenesis of the disease. To be of value in a better understanding of the anemic states a classification should be as simple as possible while still grouping together those anemias which are closely related. Erythrocytes are highly specialized cells which do not have the power of reproduction or regeneration. After circulating in the blood stream for a relatively short time, they are removed and destroyed. Any change in their number or in their structural characteristics is brought about by lesions extrinsic to the cells. Replacement, regeneration, and removal of cells from the circulation are dependent upon the hematopoietic tissues. Anemia is, therefore, only one manifestation or symptom of a disease whose primary location is in another organ or structure. A simple diagnosis of "anemia" is consequently incomplete, and although descriptive terms such as "hypochromic" and "hyperchromic" or "microcytic" and "macrocytic" give more detailed information about this symptom, they do not complete the diagnosis.

A classification based on the pathogenesis and etiology of the anemia seems preferable to one which is purely descriptive, for by this means anemias with similar backgrounds and with similar therapeutic implications will be grouped together. In some anemias more than one factor is responsible for their production, and in some the cause is still unknown. Although great strides have been made in furthering our knowledge of the pathogenesis of anemias, there are still distinct gaps to be filled, and any classification based on our present knowledge will be incomplete and subject to change.

An etiologic classification is firmly established from a historical aspect since the early grouping of the anemias into primary and secondary types was on this basis. Primary anemias included those idiopathic forms in which there was no recognizable cause. Secondary anemias were those which accompanied some obvious disease. The most prominent member of the primary group was pernicious anemia with its large and deeply staining erythrocytes, and the term *primary* was eventually applied to all anemias showing these structural characteristics. It thereby became a descriptive term rather than etiologic. *Secondary anemia* underwent a similar change in usage and it too came to be used as a descriptive term to indicate that the erythrocytes were pale and lightly staining. Although these terms are still used to some extent, it is far better to replace them with the more descriptive terms *hyperchromic* and *hypochromic*.

The erythrocytes may vary in their number, size, and hemoglobin content. These variations may be found in all combinations. Normocythemia implies a normal erythrocyte count, hypocythemia a decrease, and hypercythemia an increase in the red cell count. Erythrocytes which are normal in size are normocytic, when their size is decreased they are microcytic, and when it is increased they are macrocytic. When erythrocytes contain a normal amount of hemoglobin the cells are normochromic, when the amount per cell is decreased they are hypochromic, and when it is increased they are hyperchromic. These terms are used in various combinations in describing types of anemia. Hypochromic anemias have a low color index and a low mean corpuscular hemoglobin content, they are usually microcytic. Hyperchromic anemias have a high color index and an increased mean corpuscular hemoglobin content and are usually macrocytic. The volume index is low in microcytic anemias and high in the macrocytic type. A classification based upon these structural characteristics does not give an adequate grouping of the anemias with respect to their etiology and pathogenesis, and only vague generalizations with respect to therapy can be drawn.

The following classification is not presented as an ideal arrangement but it appears to be the most logical and has proved useful in clarifying the subject of anemia in the minds of medical students. Errors will become apparent and rearrangements will be necessary as our knowledge of the pathogenesis of the anemias is increased. The classification is based on the three fundamental causes of all anemias. (1) decreased production of erythrocytes or hemoglobin, (2) increased destruction of erythrocytes, and (3) loss of blood by hemorrhage.

CLASSIFICATION OF ANEMIAS

I. Decreased Production of Erythrocytes or Hemoglobin

A. Deficiency in the maturation factor

1. Pernicious anemia
2. Gastrointestinal lesions of certain types
3. Nutritional deficiencies—sprue and pellagra (some cases)

B. Iron deficiency anemias

1. Chronic hemorrhage
2. Idiopathic hypochromic anemia
3. Hypochromic anemia of pregnancy
4. Hypochromic anemia of infants
5. Chlorosis

C. Aplastic anemia

1. Idiopathic
2. Secondary to toxins, chemicals and radioactive substances

D. Myelophthisic anemia—mechanical interference with erythrocyte production

1. Leukemia
2. Bone marrow tumors, primary or metastatic
3. Osteosclerosis
4. Lipoid dystrophies

E. Depression of bone marrow function

1. Hypothyroidism
2. Nephritis
3. Chronic infections
4. Liver disease
5. Banti's syndrome

F. Nutritional deficiencies

1. Avitaminosis
2. Chronic diarrhea
3. Malnutrition

II. Excessive Destruction of Erythrocytes

A. Intrinsic

1. Familial and acquired hemolytic icterus
2. Acute hemolytic anemia (Lederer's anemia)
3. Sickle cell anemia
4. Erythroblastic anemia (Cooley's anemia)
5. Erythroblastosis
6. Paroxysmal hemoglobinuria

B. Extrinsic

1. Drugs and chemicals
2. Infections
3. Animal parasites and their toxins

III. Loss of Blood

A. Acute hemorrhage

B. Chronic hemorrhagic anemia

The primary function of erythrocytes and hemoglobin is the transporting of oxygen from lungs to tissues. When the amount of circulating hemo-

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degree of cardiac dilatation. There is usually a systolic hemic murmur. A venous hum may be heard in the cervical veins owing to the increased velocity of blood flow.

A severe grade of anemia is frequently accompanied by pitting edema in the subcutaneous tissues, usually noted in the dependent parts of the body. This may erroneously suggest a diagnosis of cardiac decompensation when accompanied by shortness of breath, tachycardia, and murmurs. The edema may be due in part to myocardial weakness and loss of muscle tone as a result of tissue anoxia but it is also partly due to a lowered osmotic pressure of the blood which results from changes in the plasma proteins, to alterations in the viscosity of the blood, or to increased capillary permeability.

When anemia develops in a patient with preexisting cardiac disease, the symptoms and manifestations of the latter are accentuated. When patients with sclerosis of the coronary arteries become anemic, they not infrequently develop angina of effort which, upon recovery from the anemia, disappears. In such cases the anoxia resulting from the low blood hemoglobin superimposed on that due to the impaired coronary circulation is sufficient to cause pain, whereas the vascular lesion alone was not severe enough to do so. It is doubtful if anemia will cause angina of effort when the heart is otherwise normal.

Cyanosis seldom appears in a severely anemic patient. This manifestation is brought about by a high concentration of reduced hemoglobin in the blood, and when the amount of hemoglobin is decreased, there will seldom be enough reduced hemoglobin per unit volume of blood to produce the change in color. When cyanosis does appear in an anemic patient, it is unusually significant since a nonanemic patient under the same conditions would have a far more intense degree of cyanosis.

Changes in the electrocardiogram may accompany a severe grade of anemia. These are not specific and are due to anoxia of the myocardium. They consist of changes in the electrical axis of the heart, alterations in the contour of the T wave, and occasionally in prolongation of the P-R interval. All of these become more pronounced after exercise. When the blood hemoglobin has been restored to its normal level the electrocardiographic alterations disappear, and exercise will then have no more effect than in a normal individual. Electrocardiographic changes of this type have been observed in children in whom there was no sclerosis of the coronary arteries and are undoubtedly due to anoxia of the myocardium resulting from the anemia.

A trace of albumin in the urine is commonly encountered with a severe

globin is reduced, there is a corresponding reduction in the oxygen-carrying power of the blood. The resultant anoxia of the organs and tissues of the body leads directly or indirectly to many symptoms which are common to all types of anemia. The severity of these symptoms will vary not only with the degree of anemia but also with the rapidity with which it developed. A patient in whom an anemia has been developing over a period of months will have fewer symptoms directly referable to it than will a patient with a comparable degree of anemia which has developed rapidly.

Pallor is one of the characteristic features of anemia, but the presence of pallor does not necessarily mean that the patient has anemia, nor are all anemic patients pale. Pallor may result from other causes such as peripheral vasoconstriction, edema, or myxedema. On the other hand, an anemic patient may have sufficient vasodilatation to mask the usual pallor of the skin.

Generalized muscular weakness with lassitude and fatigability is one of the common symptoms of any anemia. In severe cases it may progress to such a point that the extreme weakness and faintness on exertion will confine the patient to bed.

Symptoms referable to the gastrointestinal tract are frequently present and may consist of anorexia, nausea, constipation, or gaseous distention. These are due to the general loss of muscle tone in the intestinal tract as a result of the anoxia. It is essential, however, that a careful search be made for an underlying lesion in the gastrointestinal tract which might account not only for these symptoms but for the anemia as well.

As a result of the diminished oxygen-carrying power of the blood there is a compensatory increase in cardiac output and in the velocity of blood flow. These changes are directly proportional to the degree of reduction in the blood hemoglobin. They are accompanied by an increased heart rate which, in severe grades of anemia or when the compensatory mechanism is brought into action suddenly, produces many subjective manifestations. With moderate grades of anemia there is palpitation, tachycardia, and mild shortness of breath on exertion. The blood pressure may be slightly lowered, but the pulse pressure is high so that a throbbing sensation may be noticed by the patient. The blood is carrying sufficient oxygen in most cases of severe anemia to adequately supply the tissues when the patient is at rest, but exertion increases the demand for oxygen to such an extent that it cannot be fulfilled and dyspnea becomes evident. It is a bad omen when the anemia is so severe that air hunger appears while the patient is at rest. This is usually an indication for immediate blood transfusion. Examination reveals an increased heart rate, as well as evidences of overactivity, and a slight

PERNICIOUS ANEMIA AND RELATED MACROCYTIC ANEMIAS

PERNICIOUS ANEMIA—ADDISON'S ANEMIA, BIERMER'S ANEMIA,
ADDISONIAN PERNICIOUS ANEMIA

PERNICIOUS ANEMIA IS A MACROCYTIC HYPERCHROMIC ANEMIA WHICH IS DUE to a deficiency of the erythrocyte maturation factor, a substance necessary for the growth and development of erythrocytes. The disease occurs only in adults and is characterized by remissions, achlorhydria, mild jaundice, and evidences of sclerosis of the central nervous system.

Pernicious anemia was first described by Addison in 1849, and the name *Addison's anemia* was suggested by Biermer in 1872 when he described the condition as a "progressive pernicious anemia." A great deal has been written about the disease in the intervening years, particularly concerning the pathologic changes and theories as to its cause. Progress toward a complete understanding of the disease was slow until the effect of diet on anemia was studied by Whipple and his co-workers, and Minot and Murphy (1926) found that the ingestion of liver was a specific remedy for this type of anemia. Subsequent investigations, particularly the work of Castle and his associates and the more recent investigations on folic (pteroylglutamic) acid, have clarified and revised our ideas on the pathogenesis of pernicious anemia.

Incidence

Pernicious anemia is one of the most common varieties of severe anemia. In the University of Iowa Hospitals it accounted for 420 out of a total of 26,240 admissions to the medical service during a sixteen-year period. It is common in northern Europe and America. In Ontario, Canada, there were 15 cases per 100,000 population, and an even higher incidence has been reported from some European countries. It is seldom encountered in tropical

grade of anemia, and there may be some diminution in the ability of the kidneys to concentrate urine. In a patient whose kidneys are already damaged and nearing the verge of renal insufficiency, the appearance of anemia may be sufficient to reduce the renal function to the point at which retention of nitrogen waste products develops. Restoration of the hemoglobin to its normal level may result in a return of sufficient renal function so that the blood nitrogen drops to normal.

Cerebral manifestations are of frequent occurrence in severe anemia. A feeling of faintness on exertion or on change of position is a common symptom. Headache, vertigo, inability to concentrate, and tinnitus are also common complaints. Visual disturbances such as blurring of vision and spots before the eyes are not infrequent.

Anemia, with its accompanying train of symptoms, may complicate a great many diseases. When these manifestations become engrafted upon those of the primary illness, a complicated clinical picture may result. In some conditions, such as carcinoma of the stomach or ascending colon, many of the predominant symptoms are due to the associated anemia rather than to the primary disease.

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ministered to the patient. When beef muscle is similarly treated with gastric juice obtained from a patient with pernicious anemia, the resultant product is not effective. It appears, therefore, that some constituent of normal gastric secretion is absent from the gastric secretion of patients with pernicious anemia, and its absence interferes with normal blood formation. This gastric (intrinsic) factor interacts with some constituent of food (extrinsic factor) to produce the active principle (maturation or anti-anemic factor) which is stored in the liver. This substance effectively increases blood formation in pernicious anemia and regulates hematopoiesis in the normal person. Pernicious anemia and the allied macrocytic anemias may conceivably develop from a lack of either the intrinsic or the extrinsic factor or from poor absorption or abnormal metabolism of the maturation factor. Since the maturation factor is stored in the liver, the administration of liver or a properly prepared liver fraction to patients with pernicious anemia corrects the deficiency and hematopoiesis proceeds normally.

The intrinsic factor has not been identified, but it is not one of the recognizable constituents of gastric juice, i.e., hydrochloric acid, pepsin, rennin, or lipase. The extrinsic factor is present in a large number of substances including beef muscle, autolyzed yeast, liver, eggs, and wheat germ. It has not been isolated or identified. It contains, or is closely allied to, the vitamin B complex although they are not identical. The active principle which is present in liver and liver extract has not been identified although by purification and fractionation it can be highly concentrated in a solution free of protein and with a very low solid content. This factor can be demonstrated in normal human liver but is not present in the liver of a patient dying of untreated pernicious anemia.

The recent demonstrations of the regulatory effect of folic (pteroylglutamic) acid on erythropoiesis promise to shed more light in this field. It has been shown that this substance, in the conjugated form in which it occurs in foods, is either not as well absorbed or not as well utilized in patients with pernicious anemia as in the normal individual. Present evidence suggests that this lack of pteroylglutamic acid or a closely related substance plays an important role in the etiology of pernicious and related macrocytic anemias. Subsequent investigations in this field may unfold the mystery of their etiology.

The inherited features in this disease are achylia gastrica and an absence of the intrinsic factor. The achlorhydria is obviously not the essential factor since all cases of achlorhydria do not develop pernicious anemia.

It has been noted that the bone marrow in patients with pernicious anemia

countries. It seems to occur with about equal frequency in the two sexes although some reports indicate a slightly higher incidence in males. Seldom if ever does it occur in full-blooded Negroes. It is a disease of adult life, being extremely rare below the age of 30. About 75 per cent of the cases develop between the ages of 45 and 60, and although it is not infrequent in patients above this age, it is doubtful if true Addisonian pernicious anemia ever occurs in children.

There are definite familial and hereditary tendencies in pernicious anemia and there is an interrelationship between it and familial achlorhydria. About 20 per cent of patients with pernicious anemia will be found to have close relatives similarly affected. Wilkinson and Brockbank were able to collect from the literature a total of 139 families in which 2 or more members had the disease and 59 families in which pernicious anemia and achylia gastrica (achlorhydria plus an absence of peptic activity of the gastric juice) were present. On examining 291 relatives of patients with pernicious anemia they found 70 persons (24.1 per cent) with achlorhydria. Familial achlorhydria appears early in life as was shown by the fact that of 4 children of a father with pernicious anemia there were 3 with achlorhydria whose ages were 10, 6, and 4 years respectively. It has also been noted that idiopathic hypochromic anemia occurs frequently in those families in which pernicious anemia and achlorhydria are common.

Etiology

The older theories that have been advanced to explain the cause of pernicious anemia have been excellently summarized by Cornell. The frequency with which achlorhydria accompanied pernicious anemia was noted soon after the disease was recognized, and it was believed that this, either in itself or through its action on protein metabolism or by its effect on the intestinal flora, might be the etiologic factor. Minot and Murphy's discovery that the ingestion of liver was effective in the treatment of pernicious anemia revived the theory that it was a deficiency disease and led to further investigation along this line. Castle and his associates firmly established the relationship between the abnormal gastric secretion and pernicious anemia, and it is upon the results of their investigations that the present concept of the disease is based. They found that when the gastric secretion from a normal person is given to a patient with pernicious anemia, it produces no beneficial results, nor is benefit derived from the administration of beef muscle alone. However, a rapid regeneration of hemoglobin and erythrocytes ensues when beef muscle is incubated with normal gastric juice and the mixture is ad-

cells overshadow the more mature normoblasts and erythrocytes. Fat cells are compressed and inconspicuous. The preponderance of megaloblasts led to the concept that the bone marrow in pernicious anemia had reverted to an embryonic type of cell development. This interpretation is questioned by others who believe that the megaloblast, instead of being an abnormal or embryonic type of cell appearing only in this type of anemia, is really an early stage in the normal maturation of the erythrocyte. The preponderance of megaloblasts is believed to represent an arrest in their development at this particular stage. The degree of hyperplasia and the relative frequency of various immature forms of erythrocytes will vary in the marrow of different bones so that the peripheral blood picture cannot be correlated with the pathologic picture in any one bone. The number of leukocytes found in the marrow is variable, but leukocytes are present in greater numbers than would be expected from the degree of leukopenia which is present in the peripheral blood. It is doubtful if there is a fundamental defect in the leukoblastic tissues. The megakaryocytes are decreased in number.

The megaloblastic hyperplasia of the bone marrow as described above is found during a relapse in the clinical manifestations, at a time when the red cell count is at its lowest and when one might reasonably expect to find evidences of bone marrow inactivity rather than hyperplasia. Peabody has demonstrated by repeated marrow biopsies, at various stages of the illness in the same patient, that the hyperplasia lessens as the peripheral blood count rises and that the marrow returns to an essentially normal condition during a clinical remission. The excessive number of megaloblasts in the marrow decreases as the patient improves, and these cells are replaced by more mature normoblasts and erythrocytes as a normal and orderly erythrocytic regeneration is resumed. The amount of fat tissue in the marrow increases as the red marrow recedes to its normal limits.

These successive alterations in the bone marrow agree with the present concept of the pathogenesis of pernicious anemia, which presumes that there is a maturation arrest of the erythrocytes at the megaloblastic stage with the cells developing to this point but no farther. Since they do not mature, they are not liberated into the blood stream, and consequently an anemia develops. The bone marrow becomes overcrowded with the immature cells, and the hematopoietic marrow extends beyond its normal limits. When the deficiency of the maturation factor is corrected or when a spontaneous remission occurs, the cells progress to their adult forms, which are liberated into the blood stream. The hyperplasia decreases and the red marrow recedes to its normal volume as the anemia improves.

is hyperplastic during the height of the anemia but becomes less cellular during a remission. It is now believed that the growth and maturation of the erythrocytes are stopped at an immature megaloblastic stage because of a lack of the maturation factor which is necessary for their continued development. Maturation is resumed when this factor is supplied in the form of liver or liver extract. The erythrocytes then develop to maturity and are delivered to the blood stream. The cellularity of the bone marrow decreases as a result of the liberation of mature cells. The maturation factor appears to affect the erythrocytes at the *megaloblastic stage of their development* but has little or no effect at the slightly more mature stage of the normoblast.

Hyperbilirubinemia with a mild grade of jaundice is present during a relapse of pernicious anemia, and the cause of the disease was previously considered to be an excessive destruction of erythrocytes. One explanation for the jaundice is that erythrocytes are not being formed as rapidly as is normal and the pigments liberated by the normal erythrocytic destruction are not reutilized. As a consequence these pigments were thought to accumulate in the blood stream and produce jaundice. An excessive hemolysis of erythrocytes can be demonstrated in pernicious anemia, however, by the increase in the urobilinogen excretion in the feces. This does not appear to be the primary factor in the production of the disease although the excessive hemolysis and excessive excretion of pigment promptly cease with the onset of an induced remission.

Pathology

Although the fundamental cause of pernicious anemia is located elsewhere, the most striking histologic changes are to be found in the bone marrow. Alterations in the structure of the marrow were noted by Pepper in 1875, and Cohnheim (1876) noticed that the red marrow extended into the shafts of the long bones. The bone marrow alterations were not fully understood until the fundamental information on the production of the erythrocytes was obtained by Sabin and Doan, the *bone marrow changes were correlated with the stage of the disease* by Peabody, and the definite deficiency basis for the disease was established by Minot and Castle. Gross examination during a relapse shows the red or hematopoietic marrow to have extended beyond its normal limits and to have invaded that portion of the bone normally occupied by yellow or fatty marrow. Microscopic examination reveals that the red marrow is hyperplastic and shows a marked increase in cellularity. The hyperplasia is characterized by the presence of great numbers of immature erythrocytes in the megaloblastic stage of development. These

increasing indisposition to exertion, with an uncomfortable feeling of faintness or breathlessness on attempting it, the heart is readily made to palpitate, the whole surface of the body presents a blanched, smooth, and waxy appearance; the lips, gums, and tongue seem bloodless, the flabbiness of the muscles increases, the appetite fails, extreme languor and faintness supervene, breathlessness and palpitation being produced by the most trifling exertion or emotion, some slight edema is probably perceived about the ankles; the debility becomes extreme. The patient can no longer rise from his bed, the mind occasionally wanders, he falls into a prostrate and half-torpid stage, and at length expires. Nevertheless, to the very last, and after a sickness of, perhaps, several months' duration, the bulkiness of the general frame and the obesity often present a most striking contrast to the failure and exhaustion observable in every other respect.

The onset of the disease may be manifested by symptoms referable to the anemia itself, the cardiovascular system, the gastrointestinal tract, or the nervous system. The most frequent mode of onset is characterized by increasing pallor, weakness, fatigability, and lassitude. These progress to the point at which it is difficult or impossible for the patient to pursue his accustomed activities. The onset is usually so gradual that the patient becomes accustomed to the weakness and fatigue and, since compensatory measures developed by the cardiovascular system partially offset the effect of the lowered oxygen-carrying power of the blood, a severe grade of anemia is well tolerated. The patient is able to continue with activities which would be impossible if a similar degree of anemia had developed rapidly, and the erythrocyte count is usually very low by the time the symptoms become severe enough to bring him to a physician. The weakness and lassitude are commonly accompanied by faintness, dizziness, and tinnitus. These symptoms may progress for several weeks or months and then undergo spontaneous improvement. A history of one or more preceding episodes is frequently obtained at the time of the patient's first examination.

Cardiovascular symptoms such as occur with any severe anemia will be present when the oxygen-carrying power of the blood is sufficiently reduced by the lowering of the blood hemoglobin. This results in shortness of breath on exertion, which becomes progressively worse as the severity of the anemia increases. Palpitation and dyspnea first occur only with exertion but may progress until they are present even when the patient is at rest. Air hunger while the patient is at rest is a particularly ominous sign, immediate blood transfusion is usually indicated. The increased velocity of blood flow which occurs as a compensatory measure for the decreased oxygen-carrying power of the blood is manifested by palpitation, a throbbing sensation in the head, and frequently a venous hum which is audible over the large cervical veins. The area of cardiac dullness is increased as a result of dilatation, and a systolic murmur of hemic origin is usually present at the apex or along the left

The mucous membrane of the tongue is atrophic and the papillae are absent. The atrophy may extend into the esophagus. A similar atrophic process involves all layers of the stomach wall, only the surface epithelium remaining intact. The process is largely confined to the upper two thirds of the stomach, the pyloric region being almost uninvolved. The liver is usually slightly enlarged and shows fatty degeneration, foci of necrosis, and some periportal infiltration. Deposits of hemosiderin are prominent, especially about the periphery of the lobules. The Kupffer cells have been found to be hypertrophied in untreated patients dying during a relapse. The spleen is frequently enlarged and contains deposits of hemosiderin as well as foci of extramedullary hematopoiesis. There is a moderate amount of sclerosis, evidences of phagocytic activity are present, and the pulp contains many fragmented erythrocytes. The heart is dilated, pale, and flabby and shows fatty degeneration. No renal lesions of consequence have been observed although there may be some fatty degeneration and deposits of hemosiderin. The subcutaneous fat tissue is well preserved and is deep yellow in color.

There were lesions in the spinal cord in about 60 per cent of the autopsied patients. The characteristic lesion is subacute combined sclerosis. This is a diffuse, symmetrical degeneration involving primarily the dorsal and lateral columns of the cord in its thoracic and cervical portions. In the early stage the lesions consist of small areas of necrosis in the white matter. These enlarge and fuse together to produce more extensive damage. There is an early demyelination of the nerve fibers. Areas of degeneration interrupt the tracts, and secondary ascending degeneration results. These lesions first appear in the posterior columns but later extend to the lateral columns. Similar degenerative areas may appear in the brain. Peripheral nerves are more frequently involved than is generally believed, and sections of these commonly show degenerative changes.

Clinical Features

Pernicious anemia is a chronic relapsing disease in which the mode of onset, symptoms, and course may be extremely variable. Typical cases are not being observed throughout their entire course since the advent of specific therapy, and Addison's description, written in 1855, will bear repetition.

It made its approach in so slow and insidious a manner that the patient can hardly fix a date to his earliest feeling of that languor which is shortly to become so extreme. The countenance gets pale, the whites of the eyes become pearly, the general frame flabby rather than wasted, the pulse, perhaps large, but remarkably soft and compressible, and occasionally with a slight jerk, especially under the slightest excitement, there is an

had no soreness whatsoever. Atrophy of the tongue may occasionally occur in other types of anemia, particularly in idiopathic hypochromic anemia.

Numbness and tingling of the hands and feet is a frequent manifestation of pernicious anemia. It occurs in about 90 per cent of the patients and is the symptom of onset in 25 per cent. This subjective manifestation may or may not be associated with objective evidences of spinal cord involvement. The neural symptoms may be limited to slight symmetrical numbness and tingling or may progress to a stage of clumsiness, ataxia, and inability to use the hands for fine or coordinated work. When the lower extremities are involved, an ataxic gait develops which may be first manifested by inability of the patient to walk in the dark. This may progress to the point at which there is difficulty in walking at all times. The sense of position—kinesthesia—is lost when the posterior columns of the spinal cord are involved, and the difficulty in walking is first noticed in the dark because the visual sense is then unable to aid in properly placing the feet. The same mechanism causes the patient to “lose his feet in bed”; he is unable to tell where they are or to point to them when they are not visible. The neural involvement may progress until the patient is incapacitated and unable to care for himself and has lost control of the sphincters of the bladder and bowel.

Neurologic examination will reveal signs of involvement of the posterior or lateral columns of the spinal cord or a combination of these tracts. When the posterior columns are involved, there is ataxia, loss of vibratory sense (pallesthesia), loss of the sense of position of the extremities and of two-point discrimination, absent or diminished deep tendon reflexes, and hypotonicity of the muscles. With lateral column involvement, which is less common, there is spasticity of the muscles with hyperactive reflexes and evidences of an upper motor neurone lesion. Extensive involvement of the posterior columns may lead to a “spinal cord bladder” in addition to loss of control of the rectal and bladder sphincters. This represents one of the most serious complications of the disease since it ultimately leads to an ascending urinary tract infection, today one of the most common causes of death in patients with pernicious anemia. Peripheral polyneuritis occurs with great frequency, but its manifestations are usually overshadowed by the evidences of spinal cord involvement.

Psychic disturbances often occur. They are first apparent as minor personality changes, with restlessness and irritability predominating, but may progress to delirium, confusion, and hallucinations. Mental changes disappear during a remission so that an extremely troublesome and difficult patient may become a most pleasant and agreeable one as the blood count increases.

margin of the sternum. The pulse rate is increased. The blood pressure is slightly lowered in some patients, and the pulse wave is soft and easily compressible although a quick thrust and a poorly sustained wave are occasionally found when the peripheral resistance is low.

Gastrointestinal manifestations are common, representing the symptoms of onset in about 30 per cent of the patients. A history of prolonged dyspepsia is frequently obtained and may antedate the onset of the anemia by years. Many of these symptoms are attributable to the achlorhydria. The nature of the complaints varies, but indigestion, vague and ill defined abdominal distress, anorexia, nausea, and vomiting are particularly common. Gaseous distention is frequent; at times there may be pain of a type suggesting peptic ulcer. Recurrent attacks of diarrhea, usually mild, occur in about half of the patients at some time in the course of their illness. These too have been attributed to the achlorhydria, but it is doubtful if this is entirely responsible since the diarrhea ceases when the blood has been restored to normal even though the achlorhydria persists. Cholelithiasis and cholecystitis are unusually frequent in patients with pernicious anemia. The onset of the cholecystic disease usually precedes the anemia. Attacks of acute upper abdominal pain may occur without recognizable gallstones or gallbladder disease. These resemble the episodes of pain encountered in patients with hemolytic anemia during a hemolytic crisis but are usually less severe. The attacks have occasionally led to unwarranted laparotomy.

Soreness of the tongue occurs at some stage of the illness in over half of the patients. It is more frequent during a relapse when the anemia is severe but may appear during a remission. It is the first symptom of the disease in some patients. There may be a burning sensation of the tongue or a feeling of rawness which is aggravated by hot or highly seasoned foods. Only the tip and edges of the tongue or the entire buccal cavity may be involved. The tongue may become red and inflamed and show superficial ulcerations, but in other instances the burning sensation is present with no evidence of inflammation. After persisting for a few days or several weeks the soreness subsides only to reappear after a variable period of time. A similar burning sensation may be present in the rectum but this is less common.

Atrophy of the mucosa of the tongue is one of the common features of pernicious anemia. It is first evident at the tip and along the edges but later spreads to involve the entire dorsal surface. The papillae are atrophic, the mucosa is thin, shiny, and uncoated so that a glazed appearance is imparted to the entire surface of the tongue. Thus atrophy is not dependent upon recurrent attacks of glossitis but may be present in patients who have

appears from eight to ten years later. Its association with arteriosclerosis of the coronary arteries is of particular interest since angina of effort may occur during a relapse of the anemia but be entirely absent after the blood count has returned to normal. The electrocardiographic alterations are primarily those of coronary artery disease, but minor alterations caused by the anemia may be superimposed.

Impairment of renal function, indicated by a lowering and a fixation of the specific gravity of the urine, may result from a severe grade of anemia. When this is superimposed on a deficient renal blood flow due to arteriosclerotic changes, more serious evidences of renal insufficiency may follow. We have observed patients who developed nitrogen retention during a relapse of their pernicious anemia but whose blood nitrogen returned to normal when the anemia was brought under control. Small amounts of albumin together with hyaline and granular casts may appear in the urine as a result of the anemia. There may be a slight or moderate degree of peripheral edema as a result of changes in capillary permeability, the plasma proteins, or the altered viscosity of the blood. Water retention may occur with the onset of an induced remission so that edema appears at a time when the patient's general condition is rapidly improving.

Laboratory Features

Although the hematologic features of pernicious anemia are characteristic, the diagnosis must be based fully as much on the history and physical examination as on the blood smear. Only a tentative diagnosis can be made from the appearance of the erythrocytes; verification depends on the other findings.

The anemia is usually quite severe by the time the subjective manifestations have forced the patient to consult a physician so that an initial erythrocyte count of 2,500,000 or less is commonly encountered. Much lower values are found in many patients. Counts in the neighborhood of 1,000,000 are not at all infrequent and occasionally may drop to 500,000 or less. The reduction in the red cell count is proportionately greater than is the reduction in the hemoglobin level so that a positive color index, frequently 1.5 or over, is one of the most characteristic features of the disease. It indicates that the average hemoglobin content of the individual erythrocyte is greater than normal, a hyperchromic type of anemia. The reduction in the number of erythrocytes is also proportionately greater than is the reduction in the hematocrit—the volume of packed red blood cells per hundred cubic centimeters of blood—so that the volume index is increased, averaging about 1.4.

There is usually some loss of weight, but in spite of this the patients appear remarkably well preserved. A "lemon yellow" color is commonly present and is due to pallor combined with mild icterus. The scleras are slightly icteric rather than pearly white. A low grade irregular fever is often noted during a relapse but subsides soon after the anemia begins to improve. Small ecchymotic areas may be seen in the skin, and the patient may have bruised easily over a period of many months or years. Petechiae in the skin and conjunctiva may occur but are not particularly common. Small retinal hemorrhages are frequent in any severe anemia but are more frequent in pernicious than in other anemias of comparable severity except aplastic anemia and those complicated by thrombopenic purpura. A slight yellowish discoloration of the nerve head may also be noted on examination of the fundi. Vitiligo, irregular areas of depigmentation of the skin, is found in about 40 per cent of the patients with pernicious anemia, but the connection between these diseases is obscure as anemia, achlorhydria, and a family history of pernicious anemia are not found in patients with simple vitiligo. Areas of excessive pigmentation or of generalized pigmentation of the skin suggestive of Addison's disease may be encountered.

Gastroscopic examination reveals a picture of atrophic gastritis with a thin, pale mucosa in which the deep vessels are visualized with unusual clarity. This may be diffuse and generalized or patchy and associated with areas of superficial gastritis.

The spleen is enlarged in about 40 per cent of the patients, but the enlargement is never excessive, the organ seldom extending more than 2 or 3 cm. below the costal margin. The liver may be slightly enlarged. Both the splenomegaly and hepatomegaly disappear with adequate therapy. There is no lymphadenopathy.

Since pernicious anemia is predominantly a disease of late adult life, it is not surprising to find it associated with other diseases which are common at this age. It has been found in association with diabetes mellitus, toxic adenoma of the thyroid, myxedema, carcinoma of the stomach, and other malignant growths. The frequency of the coexistence of pernicious anemia and gastric carcinoma in the same patient has been repeatedly emphasized and although the incidence varies greatly in the different reports it appears that about 8 to 10 per cent of patients with pernicious anemia develop gastric carcinoma. It has also been found that gastric polyps occur in about 6 per cent of the patients. The frequency with which these gastric lesions complicate pernicious anemia suggests that there is some unrecognized etiologic relationship. Pernicious anemia develops first and the complicating gastric lesion

which appear rather deeply stained and have lost their central pale area because of their increased thickness. These variations in the size of the cells are best demonstrated by Price-Jones curves (Fig. 13), which portray graphically the percentage distribution of cells of different diameters. Such curves have a broad base, indicative of the wide variations between the very large and very small cells, and a peak which shows that a majority of the

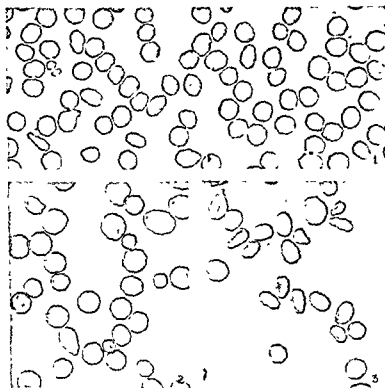


FIG. 12. Photomicrographs of normal erythrocytes and erythrocytes from patients with pernicious anemia. Above are normal erythrocytes. Below are two examples of cells from patients with pernicious anemia showing macrocytosis and variations in the size and shape of the cells.

cells are abnormally large with diameters ranging from 8.5 to 9.5 microns. Irregular and bizarre forms of erythrocytes—poikilocytosis—are encountered with great frequency.

A majority of the erythrocytes are deeply stained. In contrast to these hyperchromic cells are some which are lightly stained so that there is a great variation not only in the size and shape of the cells but in their staining reaction as well. Polychromatophilic erythrocytes are occasionally found,

This indicates that the average volume of the individual erythrocyte is greater than normal, characterizing the anemia as macrocytic in type. More detailed studies confirm the hyperchromia and macrocytosis, the mean corpuscular hemoglobin content being increased as well as the mean corpuscular volume. The greater amount of hemoglobin per cell parallels the increase in the size of the cell so that the mean corpuscular hemoglobin concentration remains about normal.

Erythrocytes of various sizes, shapes, and staining reactions are found on the blood smear, but macrocytes predominate. These are large cells, round

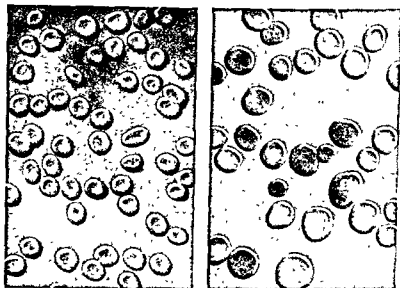


FIG. 11 Bas-relief photomicrograph of normal erythrocytes compared to those from a patient with pernicious anemia. Normal cells are on the left. On the right are cells from a case of pernicious anemia showing the greater size and thickness of the erythrocytes in this disease.

or oval in shape and deeply stained because of their increased thickness (Fig. 11). They may be 12 to 14 microns in diameter whereas the average normal red cell is 7.5 microns. Macrocytes are present in great numbers during a relapse and may comprise 30 or more per cent of the erythrocytes seen on the blood smear. In contrast to the macrocytes are many small cells—microcytes—whose diameter may be no more than 4 or 5 microns. They are less numerous than the large cells, but the contrast between the very large and the very small cells (anisocytosis) is striking and is typical of pernicious anemia (Fig. 12). Between these extremes there are large numbers of erythrocytes of normal or only slightly increased diameter, many of

are usually within normal limits although there may be a poor clot reactivity. Only in exceptional cases will the thrombopenia be associated with the other laboratory and clinical features of thrombopenic purpura.

There is an increased bilirubinemia, because of which the blood plasma is deep yellow in color, the icterus index is increased, and a positive indirect van den Bergh reaction is obtained. This is accompanied by an increased excretion of urobilinogen in the feces and, in some cases, in the urine. Bile pigments are present in the feces but not in the urine. There is a reduction in the total blood volume.

The blood picture during a remission is different from that seen during a relapse. With the onset of a remission there is a rapid increase in the number of reticulocytes followed by a rise in the red cell count and hemoglobin level. Reticulocytes are larger than normal erythrocytes so that during the time they are present in increased numbers the volume index and the mean corpuscular volume remain high. After the reticulocyte response has subsided, the evidences of increased cell volume decrease and macrocytes disappear from the blood stream. The hemoglobin level and the erythrocyte count are normal during a complete remission, and there is nothing detectable on the blood smear to indicate an abnormality of the hematopoietic system. The leukocyte count increases, and slightly immature cells may be found in the blood stream for a short time at the onset of a remission. There may be a transient eosinophilia. A sharp fall in the fecal excretion of urobilinogen occurs as the erythrocyte count rises. The jaundice subsides.

Achlorhydria is a constant feature of pernicious anemia; the number of authentic cases in which free hydrochloric acid has been found in the gastric contents is so small as to be almost negligible. The administration of histamine acid phosphate does not stimulate the secretion of hydrochloric acid in patients with pernicious anemia. Not only do they have achlorhydria but the total secretory activity of the stomach is decreased, and the volume of gastric secretion is small. Achlorhydria has been shown to antedate the anemia by many years and to persist in spite of adequate treatment with a complete clinical and hematologic remission.

The basal metabolic rate is moderately elevated during a relapse but returns to normal during a remission. The uric acid content of the blood serum may be low during a relapse but increases to normal as the anemia subsides.

The bone marrow, as found on sternal aspiration, presents features which are extremely characteristic. The most striking feature is the hypercellularity of the marrow with a predominance of megaloblasts. Cells of the erythrocytic series comprise a much higher proportion of the nucleated cells encountered

especially at the onset of a remission. These are slightly immature cells having a diffuse bluish cast of the cytoplasm. Howell-Jolly bodies, Cabot's rings, and small bits of chromatin material are sometimes found within the erythrocytes. Normoblasts are seldom encountered during a relapse although they may occasionally appear in large numbers for a short time. Megaloblasts are found infrequently although both these and normoblasts may appear at the onset of a remission. The resistance of the erythrocytes to hypotonic saline solutions is normal or slightly increased.

PRICE-JONES CURVE PERNICIOUS ANEMIA

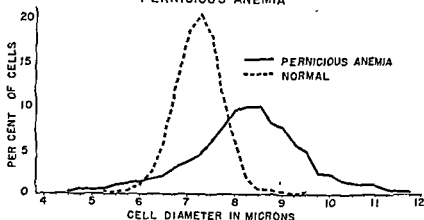


FIG. 13. The broad base of the Price-Jones curve in pernicious anemia shows the variation in the size of the cells. There are a few very small erythrocytes and a few extremely large cells having a diameter of 11 microns or more. The location of the peak of the curve shows that the greatest number of the erythrocytes have a diameter of 8.5 to 9.5 microns as compared to the curve in normal blood, in which the greatest number of cells have a diameter of about 7 microns.

A moderate reduction in the leukocyte count is characteristic of pernicious anemia, but this leukopenia is not extreme. The leukocyte count is usually in the neighborhood of 3000 to 5000. The lowering of the total white cell count is due to a reduction in the number of granulocytes. A relative lymphocytosis results, therefore, the neutrophils averaging 48 per cent and the lymphocytes 45 per cent of the total leukocyte count. There is a nuclear "shift to the right" in the neutrophils so that nuclei with four, five, or six lobules are more numerous than normal. Extremely large neutrophils with multilobulated nuclei having eight or nine lobules are occasionally encountered (see Fig. 6, p. 34).

The reduction in the number of blood platelets is proportionate to the severity of the anemia. The bleeding time and coagulation time of the blood

essential diagnostic features and still is not adequate to produce a complete remission.

If proper consideration is given to the following features of pernicious anemia, the diagnosis will rarely be in error:

From the history: (1) Remissions. A majority of the patients will have noticed one or more episodes of weakness, pallor, and icterus prior to the relapse which brought them to a physician. (2) Numbness and tingling of the extremities. This usually affects the lower extremities first and becomes most prominent in this region. It appears in about 90 per cent of the patients with pernicious anemia but may also occur in other types of severe chronic anemia.

From the physical examination: (3) Evidences of subacute combined degeneration of the spinal cord. The type of reflex and sensory changes will depend upon whether the posterior or the lateral column of the spinal cord is most severely damaged. Some evidence of organic neural lesion occurs in 40 per cent of the patients. (4) Atrophy of the tongue. This is first apparent at the tip and edges but may extend over the entire dorsal surface.

From the laboratory findings: (5) Anemia. This usually becomes severe, with an erythrocyte count of 2,500,000 or less, before the patient consults a physician unless the neurologic symptoms predominate and appear unusually early. (6) Color index above normal. This is elevated because the drop in the erythrocyte count is proportionately greater than the reduction in the amount of hemoglobin. (7) Macrocytosis. This is evident on the blood smear and also by the increased volume index and mean corpuscular volume. Variations in the size and shape of the erythrocytes are striking when the anemia is severe. (8) Leukopenia. The total white count is below 6000 in 90 per cent of the patients and in a large group of cases has been found to average slightly over 4000. (9) Jaundice. A mild grade of icterus gives the typical lemon yellow color and is accompanied by an elevated bilirubinemia. (10) Achlorhydria. A diagnosis of pernicious anemia should not be made when free hydrochloric acid is present in the gastric contents. (11) Megaloblastic hyperplasia of the bone marrow during relapse is characteristic of pernicious anemia.

A therapeutic test with a potent liver extract may be tried as a diagnostic procedure and the results of the treatment followed by a daily reticulocyte count. It must be borne in mind, however, that some other macrocytic anemias respond to this form of therapy.

There are a number of different types of anemia that may cause difficulty

than is normal and although there are more normoblasts encountered than in normal marrow, the striking features are the great increase and the predominance of megaloblasts. These are frequently encountered in clumps and in varying stages of development and mitotic figures are unusually common. The delicate chromatin network of the nuclei and their deeply basophilic cytoplasm (Chapter II) distinguish the cells. They vary greatly in size so that too much attention cannot be paid to this feature. It is the author's opinion that they represent a stage in the maturation of the erythrocyte and that the increase in their number and percentage in the marrow is the important diagnostic feature. Others believe that the megaloblast is an abnormal cell appearing only in this and related anemias. Whichever view is held, it is agreed that they are abnormally prominent in pernicious anemia. There are evidences of increased myeloid activity even though there is a leukopenia in the peripheral blood stream and abnormalities of these cells, especially in the metamyelocytes, have been described. Many of these are unusually large, stain peculiarly, and present a large irregular nucleus. The characteristic variation in the size, shape, and staining of the erythrocytes is as apparent in bone marrow smears as in the peripheral blood.

As a remission ensues, either spontaneously or as a result of therapy, the bone marrow reverts to normal. The hypercellularity diminishes, megaloblasts decrease in number and are replaced by normal erythroblasts, and the leukopoietic cells become more nearly normal in their distribution. During a complete remission the bone marrow is indistinguishable from normal.

Diagnosis

A diagnosis of pernicious anemia cannot be made with certainty from an examination of the blood alone but must depend equally upon the history, physical examination, and other laboratory procedures. Difficulty is encountered in arriving at a diagnosis in patients who seek advice because of paresthesias when the red blood count is not significantly lowered. These neurologic changes may antedate the anemia by many months. In some cases only a period of observation or a therapeutic trial with a potent liver extract will settle the problem as to whether the patient has pernicious anemia or not. Another problem is encountered in patients who have been inadequately treated with combinations of liver and iron. Under these conditions it may be difficult or impossible to tell which of the preparations were of value in producing a partial remission and which, if any, should be continued. Such therapy is frequently sufficient to obscure or cloud the

Treatment

The continuous administration of adequate amounts of the maturation factor is the fundamental principle in the treatment of pernicious anemia. This material must be given in sufficient amounts not only to maintain the erythrocyte count and hemoglobin at their normal levels but also to maintain a normal color index and cell volume for the rest of the patient's life. If normal levels are kept, the symptoms will be adequately controlled. Should symptoms recur under these circumstances, more intensive therapy is indicated. The maintenance requirements of an individual may vary from time to time. Thus, frequent clinical and hematologic observations are necessary to be sure that a relapse is not insidiously developing from inadequate treatment. The active principle of the liver extract which is administered can be stored in the liver, but in spite of this storage a relapse will begin within two months after cessation of therapy, although six months or more will elapse in most cases before symptoms reappear. A relapse may result in an extension of irreparable damage to the spinal cord. It is essential that recurrences be averted if possible. Overtreatment is far preferable to inadequate treatment, and the patient must be firmly impressed with the necessity of continuing treatment for the rest of his life.

Therapeutic Preparations

Whipple and his collaborators demonstrated that liver was the most effective food in promoting regeneration of hemoglobin in anemic dogs, and Minot and Murphy showed that a remission was induced by the feeding of liver to patients with pernicious anemia. Sturgis and Isaacs and also Sharp demonstrated that a similar effect was produced by the administration of dried hog's stomach. It was later found that the product obtained by incubating a mixture of gastric tissue and liver before drying had a greater therapeutic effect than that of either ingredient alone. Purification and concentration of liver extracts by Cohn and his associates have made available a material which is suitable for parenteral administration. The physician has at his disposal, therefore, a wide variety of preparations which contain the active principle necessary for the growth and maturation of the erythrocytes. These preparations are of value in the treatment of only those cases of anemia in which there is a deficiency of this material. They are of no value whatsoever in other types of anemia.

Whole liver, either cooked or raw, is effective in the treatment of pernicious anemia as was demonstrated in the original work of Minot and

in the differential diagnosis. Those macrocytic anemias which are closely related to *pernicious anemia* and also due to a deficiency of the maturation factor will be discussed later. *Chronic aplastic anemia* is not macrocytic nor does it present the extreme variations in size and shape of the erythrocytes which are seen in *pernicious anemia*. The bilirubin content of the blood is not elevated, spinal cord lesions do not occur, achlorhydria is not constantly present, and a more profound reduction in both the leukocytes and the platelets is present. *Familial* and *acquired hemolytic icterus* present spherocytosis, a diminished but uniform cell diameter, and a decreased osmotic resistance of the erythrocytes to hypotonic saline solutions. Spinal cord lesions are not present, and jaundice, rather than anemia, is apt to be the predominating feature. In *iron deficiency anemias* the color and volume indices are low and the erythrocytes appear hypochromic on the smear. There is no evidence of spinal cord lesions with this type of anemia. The leukocyte count is not reduced. A severe anemia accompanies *myxedema* and *chronic uremia* so that these must be considered, but they can be recognized by the proper laboratory and chemical tests. The macrocytic anemia that accompanies certain types of *liver disease* may resemble *pernicious anemia* but does not show the variability in the size and shape of the erythrocytes, the atrophic tongue, or spinal cord lesions and is usually associated with other evidences of hepatic insufficiency. *Leukemia* in an aleukemic stage may cause difficulty in diagnosis, but enlargement of the spleen or lymph nodes will usually be present, and a few immature leukocytes will be found in the blood. It may be necessary to rely on the results of sternal puncture or a biopsy of the sternal marrow to finally establish the diagnosis.

Since the patient with *pernicious anemia* requires a specific form of therapy for the rest of his life, it is essential that a diagnosis be firmly established before treatment is started. It is particularly important that the picture not be confused by the use of multiple therapeutic agents such as liver, vitamins, and iron before ascertaining which, if any, of these are needed.

Prognosis

The prognosis of properly treated and uncomplicated cases of *pernicious anemia* is excellent if therapy is continued regularly throughout life. The specific therapy is not a cure but is effective in controlling the anemia and in arresting or preventing neurologic complications. When the spinal cord lesions are already extensive, particularly with loss of sphincter control, the outlook is less favorable, and death may occur from an ascending urinary tract infection in spite of adequate treatment of the anemia.

der which has the same therapeutic action as liver extract. From 20 to 30 Gm. of this material per day is necessary to restore normal blood values. It has the same advantages and disadvantages as oral liver extract but is not available for parenteral use at the present time.

Liver-stomach concentrates are digestion products prepared from 3 parts of liver extract and 1 part of hog's stomach. By this process the potency of the liver is augmented about tenfold. Gram for gram this is the most potent product available for oral administration. Because of the effectiveness of small amounts of the material, it can be dispensed in capsules. The usual requirement during a relapse is about 6 Gm. per day.

Solution of Liver Extract for Parenteral Injection

The introduction of concentrated and purified preparations of liver extract was a distinct advance in the treatment of pernicious anemia. Intramuscular administration of these preparations has largely replaced the oral route. The effects of the treatment become evident more quickly, absorption is certain, nausea and vomiting do not interfere with its action, and the expense of treatment is less than with orally administered extracts.

There are many preparations on the market, the strength of which varies from 1 unit per 10 cc. to 15 units per 1 cc. The physician must be certain of the strength of the solution he is using as well as being sure that it is a tested and approved product. The preparations are given intramuscularly, and it is best to inject them deep in the gluteal muscles, particularly when the less concentrated preparations requiring a large volume of fluid are used. There is less pain and distress following the injection of 1 cc. of the concentrated extract.

The amount of liver extract required will vary from patient to patient and will depend upon the severity of the anemia. In an uncomplicated case with an erythrocyte count of about 1,000,000 a satisfactory outline of treatment is as follows: 15 units (1 cc. of the concentrated solution) of liver extract daily in the muscle for the first seven days, and then 15 units twice a week until the blood count has returned to a near normal level. The maintenance dose must then be determined for each patient. A majority of the patients will require 15 units every two or three weeks. In less severe cases smaller amounts of the extract will suffice to bring the blood to normal. If a less concentrated preparation is used, a larger volume of the material will be required. The dosage advised by the manufacturer of the extract and printed on the original package is usually satisfactory. The final criterion which determines the amount of liver extract required is the condition of the patient and his re-

Murphy. Subsequent reports have shown that remissions of many years' duration can be maintained by this means without the use of liver extracts. Adequate treatment requires the ingestion of from $\frac{1}{2}$ to $\frac{3}{4}$ pound of liver per day. So-called "liver cocktails" are prepared by grinding raw liver finely, passing it through a sieve to remove the coarse particles, chilling the preparation, and mixing it with chilled fruit juice of any type. Most patients will tolerate this preparation without complaint for longer periods of time than cooked liver. Whole liver is less effective, less reliable, and more expensive than more concentrated products.

Liver extracts vary in form, potency, and concentration. All of these products formerly carried on their labels a statement of the amount of whole liver from which a specified amount of the extract was derived. Such a basis for standardization became inadequate with greater concentration and refinement of the extracts, as some of the original active principle is lost in this process. The Anti-Anemia Preparations Advisory Board of the United States Pharmacopeia defined a "unit" of liver extract in order to better evaluate these products. The unit is designated as that amount of material which, when given daily to a patient with pernicious anemia, will produce a satisfactory reticulocyte rise and a satisfactory increase in the number of red blood cells and hemoglobin. The effectiveness of liver extract varies with its mode of administration, the oral route requiring at least thirty times as much of the active principle as is necessary to produce a similar response by parenteral administration. The amount of liver extract constituting a unit is different for each route of administration. In treating a patient it is essential that a product approved by the Council on Pharmacy and Chemistry of the American Medical Association be used so as to insure a potent preparation.

Liver Extract for Oral Administration

Liver extract may be obtained in either powdered or liquid form. The strength varies so that 1 oral unit in a dry preparation may be present in from 4.5 to 25 Gm. of material, and in the liquid state 1 unit is present in from 45 to 60 cc. of the solution. These preparations provide a distinct advantage over whole liver, but even in these forms it is difficult for many patients to take a sufficient amount of the material. The taste is not pleasant and may cause nausea or vomiting, which prevents adequate absorption. The oral route cannot be depended upon to supply adequate amounts of the active principle in severe or complicated cases so that preparations for oral administration are less efficacious than those given parenterally.

Desiccated and defatted hog stomach (ventriculin) is a dry granular pow-

extract. The reticulocytes increase in number rapidly to reach a peak on the seventh to the tenth day, following which they subside to a normal level. The height of the reticulocyte response varies inversely with the erythrocyte count at the onset of therapy so that the greatest response is obtained in those having the lowest initial erythrocyte count provided there are no complicating factors. A reticulocytosis of from 50 to 70 per cent may be

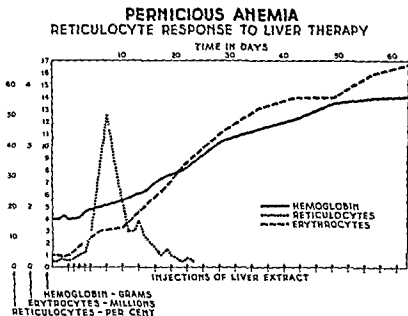


FIG. 14. The response of the reticulocytes, erythrocytes, and hemoglobin to liver extract in a patient with pernicious anemia.

expected with an erythrocyte count of 1,000,000 or less. In the accompanying table the United States Pharmacopeia Advisory Committee suggests the reticulocyte response at different erythrocyte levels as proof of the potency of a liver extract.

Initial Red Cell Count (Millions)	Reticulocyte Response (Per Cent)
1.0	41.8
1.5	28.4
2.0	18.6
2.5	11.1
3.0	5.1

A greater reticulocyte response may reasonably be expected

sponse to therapy. There is no danger and no harm in overtreatment; it is our practice to administer doses in excess of 1 unit per day although the latter should be sufficient to produce a remission.

The maintenance dose must be determined for each patient individually, and no specified amount of any of the preparations can be predicted beforehand as the requirement. The presence of an infection or of advanced arteriosclerosis will necessitate a larger dose than is necessary in the absence of these complications. Subacute combined sclerosis of the spinal cord is also an indication for more intensive treatment. Sufficient material should be given to maintain a clinical and hematologic remission and to prevent or check the development of neural lesions. The treatment must be continued for life. It is inadvisable to attempt to give the smallest possible amount of liver extract that is necessary to keep the red cell count at normal. A little generosity in this respect will pay dividends by preventing recurrences.

The advisability of using a highly purified preparation in those patients with neural involvement has been questioned. It is possible that something of benefit to nerve tissue has been removed in refining the product. For this reason some physicians have advised that patients with neural involvement receive a less concentrated product, such as one containing 2 units of liver extract in from 3 to 10 cc. of solution. From 2 to 12 units of this preparation may be given daily, depending upon the severity of the anemia. This treatment may be augmented by whole liver, crude liver extracts, or ventriculin by mouth or by injections of thiamin chloride. Recent observations covering a period of several years have failed to show any significant difference in the effect of crude and highly purified extracts on the neural lesions. It seems doubtful if there is any advantage in using crude extracts in these cases, but reliance should not be placed on oral preparations in patients in whom there is extensive neural involvement.

Although the maturation factor is stored in the liver following its administration in the form of liver extract, it is not practical to give exceedingly large amounts at infrequent intervals either as a curative or as a maintenance dose.

Results of Treatment

Subjective improvement will be noted by the patient soon after treatment is begun and before there is an appreciable increase in either the hemoglobin or the erythrocyte count. The first manifestation of improvement in the blood is an increase in the number of reticulocytes. This will be apparent within twenty-four to forty-eight hours after the first injection of liver



PLATE VII

doses of the extract. After reaching its peak the number of reticulocytes drops rapidly to the normal level where it remains even though the erythrocyte count increases rapidly (Fig. 14).

During the time that reticulocytes are present in greatest numbers there is no apparent increase in the erythrocyte count, but soon after they return to their normal level the red cell count begins to climb. The rise in the cell count is correspondingly more rapid than is the increase in hemoglobin so that the color index may drop below normal at this time. The erythrocyte count progresses irregularly with periods of rapid increase followed by periods in which it remains stationary. It requires from eight to fifteen weeks for the normal level to be reached. Subjective improvement is apparent before there is an appreciable increase in the red cell count, and the patient feels surprisingly well by the time the count has reached 3,500,000.

The leukocyte count rises rapidly, and a few immature cells of the granulocytic series may occasionally appear in the blood stream. An eosinophilia frequently occurs at the onset of a remission but is more frequent and more pronounced with the use of raw liver than with liver extracts.

A sharp spike of fever occasionally accompanies the reticulocyte crisis but quickly subsides to normal. The mild febrile reaction, which is common during a relapse, disappears after the reticulocyte response has subsided. A slight dependent edema may appear during therapy or when the patient first gets out of bed.

Less rapid improvement is noted in patients with extensive neural involvement, but Strauss has shown that complete arrest of the neural manifestations may be expected with adequate therapy. If such manifestations have not appeared, they may be entirely prevented. The subjective manifestation of numbness and tingling may completely disappear unless there is extensive involvement of the spinal cord. The objective manifestations in the reflexes

PLATE VII.

Blood smear from a patient with pernicious anemia, stained with Wright's and cresyl blue, showing the increase in the number of reticulocytes which occurs with adequate therapy. This indicates an increased activity of the erythropoietic elements of the bone marrow. (Sturgis and Isaacs, Pernicious anemia In Downey's Handbook of Hematology. Paul B Hoeber, Inc.)

and sensations improve more slowly or not at all. It is only by intensive and continuous therapy that their progress can be arrested.

The urobilinogen content of the feces drops precipitously when treatment is begun. The jaundice soon disappears. All evidence of excessive hemolysis of the erythrocytes subsides. Rhoads has suggested that the active principle which is contained in liver extract neutralizes some hemolytic agent which has been present in the blood.

Unpleasant reactions will occur with injections of liver extract in a few cases. These may consist of urticaria, localized redness and swelling at the site of injection, or generalized reactions which may be so severe that it is necessary to discontinue parenteral injections. A change to a different brand of liver extract or to a preparation of different concentration will sometimes prevent these reactions. It may be necessary to change to the oral route of administration if the reactions persist.

Achlorhydria persists in spite of adequate treatment, the return of free hydrochloric acid having been noted in only three cases.

In a few cases the hemoglobin increases satisfactorily to a certain level and then remains stationary even though the erythrocyte count continues to rise. This apparently results from exhaustion of the available supply of iron. The hemoglobin will eventually resume its upward trend, but the administration of a simple iron salt at this time will hasten its regeneration (Fig. 15). This does not mean that iron should be given routinely to all patients with pernicious anemia. It is necessary in only a few selected cases.

Patients with pernicious anemia should be encouraged to be out of bed, to move about, and to use their extremities as much as their condition will permit. Such activity is advantageous in combating the extension or appearance of subacute combined sclerosis.

Transfusions are seldom required. The response to intramuscular liver extract is so prompt that other measures are seldom necessary even though the red cell count is extremely low. A transfusion should be given immediately, however, if orthopnea or air hunger is present while the patient is at rest or if stupor or a semicomatose state develops. It is advisable to type and cross match the blood of patients having a red cell count below 1,000,000 so that a donor will be available immediately if a transfusion becomes necessary.

The administration of hydrochloric acid does not influence regeneration of blood but it may relieve the gastric distress or diarrhea in certain patients. It can be administered in doses of 2 to 8 cc. of the U. S. P. dilute hydrochloric acid in water with each meal.

The diet should contain a liberal amount of meat, vegetables, and fruit. It is advisable to include liver in the diet at least once a week, but this is not necessary if it is distasteful to the patient. Regulation of the diet is not particularly important except for maintaining general health since the specific requirements for hematopoiesis are being filled by the liver extract.

Suitable physical therapy should be given to those with extensive neural involvement. This consists of massage and passive motion and, if necessary, the use of specific exercises for retraining muscles.

Folic Acid, Pteroylglutamic Acid

Through a long series of researches by many groups of investigators there has been discovered a member of the vitamin B complex which has a profound effect upon erythropoiesis and which may ultimately lead to a better understanding of the pathogenesis of pernicious anemia and the related macrocytic anemias. Its place in the treatment of pernicious anemia is not entirely clear at the present time but the first wave of enthusiasm for its use in this disease has subsided.

It was shown in 1940 that a substance obtained from plant or animal tissue was necessary for the growth of certain bacteria. The test organism used was *Lactobacillus casei* and the substance necessary for its growth was called the *L. casei* factor. It was present in certain fractions of liver and later was obtained in highly concentrated form from spinach; because of this source it was called "folic acid." Earlier work had shown that a macrocytic type of anemia with leukopenia could be produced in monkeys which were fed a deficient diet and that this anemia could be corrected by feeding a yeast extract (marmite). The substance necessary to relieve the anemia in these animals was called vitamin M. Subsequent investigations showed that the anemia and leukopenia in these monkeys could also be corrected by the *L. casei* factor thereby demonstrating a relationship between folic acid and vitamin M.

Another group of investigations on a nutritional deficiency in chicks which could be corrected by feeding yeast, middlings, alfalfa, or wheat bran was reported and it was noted that in addition to other features there was a macrocytic hyperchromic anemia. The substance necessary to correct these changes was termed vitamin B₁₂ and subsequent studies demonstrated an interrelationship between vitamin B₁₂ and folic acid. A crystalline compound was isolated from liver which corrected the deficiency in chicks and was also highly active as a growth factor for *L. casei*. In 1945 a synthetic compound was prepared which was believed to be identical with the crystalline *L. casei* factor previously isolated from liver.

Those mixtures of liver, iron, and vitamins which are so prevalent on the market today have no place in the treatment of pernicious or any other type of anemia. Many of these do not contain an adequate amount of liver

PERNICIOUS ANEMIA

EFFECT OF IRON AND AMMONIUM CITRATES 6 GRAMS DAILY

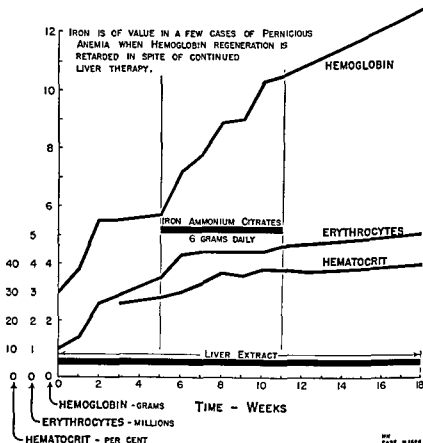


FIG. 15. Showing the response which is obtained with the administration of an iron salt in addition to liver extract in certain selected cases of pernicious anemia.

extract, and the other ingredients are unnecessary in a vast majority of patients. Vitamins and iron can be administered separately in known and adequate amounts if they are necessary. If these shotgun mixtures are administered before a diagnosis is firmly established, they may confuse the clinical picture so that the future course of treatment cannot be accurately outlined

prevent the development or progression of the neurologic symptoms of the disease and we have observed, as has Heinle, an almost explosive progression of the subacute combined degeneration of the cord even while the patient was receiving folic acid in what is presumed to be an adequate dosage and in whom the hematologic features of the disease were adequately controlled.

Because of its failure to control the neurologic features of the disease folic acid cannot be recognized as adequate therapy for pernicious anemia and cannot replace liver extract. In its present state the use of folic acid is dangerous because the neurologic complications may develop rapidly while the patient is receiving supposedly adequate treatment. It is to be hoped that a satisfactory potent oral preparation may be found in the course of present investigations with these compounds.

Vitamin B₁₂ has recently been crystallized from liver extract and extremely minute amounts have proven to be effective in treating pernicious anemia and in controlling the neurologic features.

MACROCYTIC ANEMIAS CLOSELY ALLIED TO PERNICIOUS ANEMIA

The relationship between the gastrointestinal tract and erythropoiesis has been firmly established by the experimental studies on pernicious anemia. It is generally believed that the gastric (intrinsic) factor acts upon certain constituents of food (extrinsic factor) to produce an antianemic or maturation factor which is absorbed from the gastrointestinal tract, stored in the liver, and released to the bone marrow for utilization. The intrinsic factor is lacking in pernicious anemia and as a result the antianemic principle is not produced in adequate amounts. It is obvious that defects or deficiencies may occur at other stages in the production of this substance. There may be a deficiency of the extrinsic factor from an inadequate diet which will likewise interfere with the production of the maturation factor. Absorption of the maturation factor from the gastrointestinal tract may be inadequate, or the storage and distribution of the material by the liver may be disturbed in such a way as to interfere with its utilization. A macrocytic anemia which simulates pernicious anemia results if there is a disturbance at any stage in the production of the maturation factor.

Sprue

Sprue is a chronic relapsing disease characterized by glossitis, indigestion, diarrhea with voluminous frothy stools, and an anemia which is usually of a hyperchromic, macrocytic type. It is common in tropical countries and

Various investigators had shown that when rats were fed a highly purified diet to which succinylsulfathiazole, sulfaguanadine, or sulfasuxidine was added there developed an agranulocytosis, leukopenia, anemia, and hypocellularity of bone marrow. These hematologic changes could be prevented or cured by the use of crystalline *L. casei* factor.

In the human it had been observed that the macrocytic anemia of pregnancy as well as tropical macrocytic anemia responded to the administration of a yeast extract or a crude liver extract. It had also been shown that pernicious anemia responded to the administration of dehydrated brewers' yeast but that there was a difference in the response of pernicious anemia and the anemia of pregnancy. Various other types of macrocytic anemia had been studied and it was noted that several of these responded to oral or crude liver extract but not to the highly purified form that was effective in pernicious anemia. Since folic acid had been found to be effective in the nutritional macrocytic anemia of monkeys it was tried in certain cases of macrocytic anemia in the human. A prompt hematologic response was obtained in nutritional macrocytic anemia, Addisonian pernicious anemia, and the macrocytic anemia of tropical and nontropical sprue. It is recognized at the present time that folic acid is effective in restoring the hematopoietic system to a normal function in those macrocytic anemias which are characterized by a megaloblastic bone marrow. Microbiologic assay has shown that the blood of man contains 2 micrograms of free folic acid per 100 cubic centimeters. The effect of diet on this free folic acid level has not yet been demonstrated.

Folic acid, chemically, is pteroylglutamic acid and is closely related to nucleic acid and is either identical, or closely allied to vitamin B₁₂ and vitamin M. The exact mechanism of its mode of action is still unknown but it is possibly a part of the enzyme system of the body. It has been suggested that the conjugated form of folic acid, as it is found in foods, is not properly absorbed or utilized by certain individuals and in its absence maturation of erythrocytes does not proceed normally. Folic acid is not the active principle of liver extract nor is it the intrinsic or extrinsic factor of Castle.

Folic (pteroylglutamic) acid is effective in bringing about normal erythropoiesis in pernicious anemia. With its administration there is a reticulocyte response identical to that obtained with liver extract, the hemoglobin and erythrocyte levels increase to normal, the bone marrow returns to its normal state, the appetite improves and symptomatically the patient improves. This hematologic remission can apparently be maintained indefinitely with folic acid in a majority of patients although at the present time it appears that a few patients relapse in spite of this treatment. Folic acid does not improve nor

The hematologic changes in sprue are not constant but in most cases, particularly in adults, there is a macrocytic anemia which is indistinguishable morphologically from pernicious anemia. It is associated with leukopenia and thrombopenia. In some instances, however, the anemia is microcytic and hypochromic in type. Achlorhydria, which is constantly present in pernicious anemia, is found in about 30 per cent of the patients. The sternal marrow in those cases showing a macrocytic anemia reveals a megaloblastic arrest similar to that encountered in pernicious anemia.

Treatment

The most effective method of therapy is folic (pteroylglutamic) acid. A dosage of 20 mg. per day by mouth is effective although it has not been determined as yet whether or not this is the minimum effective dose. It has been shown that folic acid in this amount produces a prompt regeneration of the blood and a return of the alimentary tract to normal function. An improved sense of well being appears within a few days after the onset of treatment; the glossitis is relieved, regeneration of the lingual papillae is soon evident, and the diarrhea ceases. There is a gain in weight, an increased appetite, and an improvement in the glucose tolerance curve. A reticulocyte response occurs and there is an increase in all of the formed elements of the blood. The cellular elements of the bone marrow return to their normal distribution. Folic acid, in conjunction with a proper diet, appears to bring about a complete and lasting remission in sprue and to control many of the manifestations of the sprue-like syndrome found in certain types of gastrointestinal lesions. We have observed one case of a sprue-like syndrome, which followed a short circuiting operation on the intestinal tract, in which the anemia, weakness, diarrhea, and weight loss were adequately controlled by this drug.

The administration of liver or a crude liver extract has heretofore been the accepted and effective method of treatment of this disease and the response has been similar to that described for folic acid. The liver extract should be given intramuscularly rather than by mouth in order to insure absorption in the presence of diarrhea. The treatment must be more intensive with sprue than with pernicious anemia. The highly purified and refined liver extracts are of little value; far better results are obtained with the less concentrated forms. The therapeutic results are excellent when treatment is begun early, but in the long-standing, far-advanced cases little can be accomplished. Brewer's yeast or autolyzed yeast should also be given.

The diet, particularly after the severe gastrointestinal manifestations have subsided, should be low in fats and carbohydrates but high in vitamin and

has been termed "tropical" sprue in contradistinction to "nontropical" sprue, which is found in more temperate climates. The two conditions are probably identical.

The exact etiologic factor of sprue is unknown although it is undoubtedly a nutritional deficiency and can be reproduced in experimental animals by means of inadequate diets. There appears to be a deficiency of the extrinsic factor in the diet of persons with sprue and perhaps an inadequate absorption of the antianemic factor from the intestinal tract. The pathologic changes encountered in sprue are somewhat variable, but atrophy of the walls of the gastrointestinal tract is common and may lead to dilatation of the colon. In patients having a severe macrocytic anemia the bone marrow is hyperplastic and presents a picture similar to that found in pernicious anemia. The mechanism whereby folic acid enters into the production of this anemia is not clear as yet but it has been suggested that it may be due to improper absorption or an abnormal metabolism of this vitamin.

Clinical Picture

The onset of the disease is usually insidious with a prolonged period of indigestion, recurring attacks of diarrhea, flatulence, fatigability, listlessness, and irritability. In a few patients the onset is abrupt. The predominant features throughout the entire course are referable to the gastrointestinal tract. Glossitis with burning and rawness of the buccal mucosa is a prominent symptom. Not infrequently a similar sensation will be noted in the rectum and vagina. This is usually intermittent, and the inflammation and ulceration of the mucous membranes may spontaneously subside. Epigastric distress with attacks of severe abdominal pain is of common occurrence. Gaseous distention may be particularly troublesome. Diarrhea is the most characteristic feature of the disease, but in the early stages there may be only a single, copious, frothy, fatty stool each day. This is usually passed in the early morning. As the disease progresses, the stools become more numerous, and with the continued diarrhea the patient loses weight and becomes extremely emaciated and cachectic. Although paresthesias of the hands and feet are common, only rarely do evidences of degeneration of the spinal cord appear. Pallor, glossitis, and emaciation are the principal findings on physical examination. Roentgenologic examination of the small intestine reveals a deficiency pattern or "moulage" sign in a high percentage of the cases. The characteristic features of this are best demonstrated by means of a motor meal. A flat oral glucose tolerance curve is a characteristic finding due to impaired absorption. A low blood cholesterol level may be present and in some a hypoproteinemia

or it may be hypochromic. When the former type is present, it is probably the result of a deficiency of the extrinsic factor.

Lesions of the skin are among the most important of the clinical manifestations. They consist of a symmetrical dermatitis on the exposed surfaces or on irritated areas of the body. They are first erythematous in type but progress to darkened roughened areas with vesicles and bullae. Repeated exacerbations lead to thick, rough, pigmented areas or to atrophy of the skin. Glossitis and stomatitis occur early in the disease, and the tongue becomes red, swollen, and ulcerated. Abdominal distention and discomfort are common; in the later stages diarrhea becomes a particularly distressing feature. Nervous, and particularly mental, symptoms are a part of the characteristic syndrome.

Treatment

Nicotinic acid is spectacular in its effectiveness in the treatment of pellagra but should be supplemented by a high protein and high caloric diet. Oral administration of liver extract and yeast has been shown to be therapeutically effective, but parenteral injections of the less refined liver extracts are more beneficial than is liver extract by mouth. Folic acid, 20 mg. per day, has also been demonstrated to be effective in producing a hematologic remission when a macrocytic anemia complicates this disease.

Tropical Macrocytic Anemia

Tropical macrocytic anemia is a condition occasionally observed in tropical countries, most frequently in India. It may develop as a complication of other diseases such as malaria, syphilis, tuberculosis, and hookworm infestation, but it has also been reported as a separate disease entity unassociated with infections or infestations. It occurs in either sex, but Wills has found it more frequently in women, particularly as a complication of pregnancy.

It seems doubtful if this condition exists as a separate disease entity. Malaria, nutritional deficiencies, intestinal parasites, and unhygienic living conditions are prevalent in the regions from which the disease has been reported, and it is difficult to exclude these as etiologic factors. They undoubtedly are the cause of the anemia in most, if not all, of the patients.

The anemia is macrocytic and hyperchromic in type. The hematologic picture is identical to that found in pernicious anemia, there being a reduction of the platelets and leukocytes as well as of the erythrocytes. The symp-

protein content. The diet, with the additional yeast, may be sufficient to control the symptoms so that liver extract or folic acid may be discontinued after a remission is brought about. In some cases, however, the liver extract or folic acid must be continued indefinitely. A high calcium intake is advisable in chronic cases and if the serum calcium is low 15 cc. of a 5 per cent solution of calcium chloride may be given intravenously. Calcium lactate or gluconate may be given by mouth in doses of 1 Gm three times daily.

Nontropical Sprue

Nontropical sprue, idiopathic steatorrhea, and celiac disease are terms which have been applied in temperate climates to a condition resembling tropical sprue. The present consensus is that these conditions are identical to the tropical form and that all of them are manifestations of the same disease. The differences in the clinical manifestations of tropical and nontropical sprue are primarily in intensity. In the temperate climate where the disease is less prevalent the cases are apt to go unrecognized and untreated. As a result, the manifestations become more severe with irreversible changes occurring in the gastrointestinal tract. Metabolic disturbances are likely to be prominent in the advanced stages of the disease so that a lowered serum calcium accompanied by tetany is common. The serum proteins are frequently decreased to such a degree that edema develops. The hematologic features are the same as in tropical sprue, and either a hyperchromic or a hypochromic anemia may be encountered.

The treatment is the same in nontropical as in tropical sprue, but since the cases of nontropical sprue are frequently unrecognized until they have reached a far advanced stage, the results of therapy are less satisfactory.

Macrocytic Anemia of Pellagra

Pellagra is a nutritional deficiency which occurs as a result of an inadequate diet or because of inadequate absorption of food from the gastrointestinal tract. It is characterized by lesions of the skin, alimentary tract, and nervous system. Nicotinic acid has been found to be a specific remedy for many of the symptoms of pellagra, but there is also a deficiency of other fractions of the vitamin B complex. The disease is not a result of a deficiency of nicotinic acid alone.

Anemia is not an outstanding feature of pellagra. When it is present it may be either a macrocytic hyperchromic type which resembles pernicious anemia

hydia or hypochlorhydia was found in 9. Pernicious anemia of pregnancy has been rare in our experience, only 4 such cases being encountered among 11,370 admissions to the obstetrical service. Achlorhydia has been present in all the cases we have observed, but there has been none with lesions in the spinal cord.

Treatment

The treatment is the same as for Addisonian pernicious anemia. Folic acid has been shown to be effective in doses of 20 mg. per day. Liver extract is likewise effective but a more intensive course of liver extract is advisable and it should be continued throughout the pregnancy. In some of the patients the administration of an iron salt seems helpful. There is no tendency for these patients to have a relapse after delivery although recurrences may develop with subsequent pregnancies. If the hemoglobin and erythrocytes have been brought to their normal level, there is no necessity of continuing treatment after delivery. If the patients become pregnant again, it is advisable to give liver extract prophylactically during the period of gestation. Fifteen units (1 cc. of the concentrated extract) intramuscularly every two weeks should suffice for prophylaxis. The dose should be increased if macrocytosis or anemia appears.

The cause of pernicious anemia of pregnancy has been variously ascribed to a dietary deficiency in which there is a lack of the extrinsic factor, to a temporary inhibition or absence of the intrinsic factor in the gastric contents, or to a depletion of the maternal supply of the maturation or anti-anemic factor owing to the fetal requirements. A combination of these causes may be present. It is of interest to note that these patients develop a macrocytic anemia only during pregnancy and not with other metabolic disturbances or illnesses.

Anemia of Liver Disease

A macrocytic anemia is frequently encountered in patients with severe and long-standing liver damage. Since the liver is the principal depot for storage and release of the antianemic factor, it is reasonable to assume that disease of the liver might interfere with these functions and so produce an anemia. The anemia is normocytic or macrocytic in type when it is not complicated by hemorrhage. Wintrobe found that a macrocytic anemia did not occur with minor lesions or with acute necrosis of the liver but was most frequently encountered with diffuse involvement such as portal cirrhosis.

toms consist of soreness of the mouth and tongue, diarrhea, edema, weakness, and fever in addition to the symptoms referable to the anemia. Degenerative changes in the spinal cord are not present, and achlorhydria is rare. Jaundice is seldom encountered. The bone marrow presents a megaloblastic hypercellularity similar to that of pernicious anemia.

The disease is fundamentally a dietary deficiency in which proteins, fats, and vitamins are low. Although it is frequently encountered during pregnancy, this is merely an exciting or contributing factor. This anemia can be cured by the use of folic acid in a dose of 20 mg. per day in conjunction with an adequate diet. It also responds to the administration of a crude liver extract. Autolyzed yeast is also an effective therapeutic agent. The diet should be high in proteins, fats, and vitamins. The results obtained with crude liver extract in this type of anemia and the absence of a response to the concentrated forms demonstrate that the crude extracts contain some hematopoietic factor not present in those which are more highly refined.

Pernicious Anemia of Pregnancy

Hematologic studies on women during pregnancy show a steady decline in the hemoglobin concentration and erythrocyte level as pregnancy progresses. When these findings are correlated with the changes in blood volume, they indicate not a true anemia but merely a "physiologic anemia" resulting from hydremia. The blood returns promptly to its normal level following parturition. In addition to this physiologic change there are many women with various types of anemia who become pregnant. In such patients the pregnancy is coincidental and is not the cause of the anemia. Any type of anemia becomes worse during pregnancy.

A true anemia develops during pregnancy in many women. In this group of patients the pregnancy is the exciting factor and, at least indirectly, the cause of the anemia. An anemia of the hypochromic, iron deficiency type is the most common and will be discussed later. An additional group of patients develops a macrocytic hyperchromic type of anemia which has been termed "pernicious anemia of pregnancy." Strauss reported 10 such cases in which the hematologic picture was identical to that of pernicious anemia, there being a positive color index, macrocytosis, and a reduction in the number of leukocytes and platelets. The clinical manifestations are more severe than with a hypochromic type of anemia, and gastric distress, diarrhea, and soreness of the tongue are common complaints. Combined degeneration of the cord was present in only 1 of Strauss's patients, but achlor-

Gastrointestinal lesions

Structures	Number of Cases
Small Intestine	
Tuberculous	11
Nontuberculous	6
Not specified	8
Total	25
Large Intestine	
Tuberculous	1
Nontuberculous	2
Carcinoma	1
Postoperative	1
Not specified	2
Total	7
Anastomoses	
Entero-enterostomy or enterocolostomy	13
Gastrojejunocolic fistula	4
Gastrocolic fistula	1
Total	18
Diverticulosis	$\frac{1}{1}$
Total	51

These patients may present not only the hematologic picture of pernicious anemia but many of the other clinical features as well. Glossitis, icterus, paresthesias, and occasionally evidences of organic involvement of the spinal cord have been observed. The macrocytic anemia is apparently due to inadequate absorption of the antianemic or maturation factor from the intestines. The bone marrow presents a picture similar to that of pernicious anemia.

Treatment. The primary feature in the treatment of such cases is to relieve the obstruction and to get rid of the areas of stasis. The administration of folic acid or a potent liver extract leads to improvement of the anemia. This is of value in the preoperative treatment in some cases and in controlling the anemia of those patients in whom short-circuiting operations have been done.

Anemia of *Bothriocephalus Latus* Infestation (Fish Tapeworm)

An infestation with the fish tapeworm will occasionally cause a macrocytic anemia which is indistinguishable from pernicious anemia in so far as the hematologic and clinical features are concerned. The incidence of this type of anemia is not high even among those known to be harboring the parasite. Infestation with the fish tapeworm is common in Japan and in

The administration of liver extract is effective in controlling this anemia in some patients whereas others fail to respond. It is advisable to give liver extract an adequate therapeutic trial in such patients, but there is no value in its continued use if the anemia is not definitely improved. Folic acid should also be tried in such cases although definite proof of its effectiveness has not been demonstrated as yet.

Macrocytic Anemia with Lesions of the Gastrointestinal Tract

Gastric Lesions

Pernicious anemia or an anemia with identical hematologic characteristics has been observed with a wide variety of lesions of the gastrointestinal tract. Such an anemia is not infrequently encountered in patients who have had a total or partial gastrectomy although it does not develop in all patients who have had this type of operative procedure nor does it always occur in experimental animals after removal of the stomach. If the gastric secretion or the mucosa of the stomach were the only source of the intrinsic factor, one might expect a macrocytic anemia to occur invariably after total gastrectomy. *The fact that this does not happen lends support to the contention that this factor is also formed in the duodenum or elsewhere in the intestinal tract.*

We have observed one patient in whom a hypochromic microcytic anemia was present prior to a partial gastrectomy and who presented, three years later, a typical hematologic picture of pernicious anemia with characteristic macrocytic and hyperchromic erythrocytes, leukopenia, thrombopenia, and mild jaundice—all of which completely subsided with the administration of liver extract. Hematologic changes of this type occur not only after gastric resection but occasionally with pyloric stenosis, gastric polyps, carcinoma of the stomach, and other lesions which interfere with the formation of the intrinsic factor. Liver extract or folic acid is of value in combating the macrocytic anemia produced in this manner

Intestinal Lesions

A macrocytic anemia may accompany a wide variety of intestinal lesions. It is apt to occur with those conditions which produce partial obstruction and stasis, with fistulas, or following anastomotic surgical procedures in which there is stasis in the loop of the intestinal tract. Such an anemia occasionally occurs in association with regional ileitis, probably as a result of the stasis. Barker and Hummel present the accompanying table to show the various lesions which are known to have produced this type of anemia

Gastrointestinal lesions

Strictures	Number of Cases
Small Intestine	
Tuberculous	11
Nontuberculous	6
Not specified	8
Total	25
Large Intestine	
Tuberculous	1
Nontuberculous	2
Carcinoma	1
Postoperative	1
Not specified	2
Total	7
Anastomoses	
Entero-enterostomy or enterocolostomy	13
Gastrojejunocolic fistula	4
Gastrocolic fistula	1
Total	18
Diverticulosis	$\frac{1}{51}$
TOTAL	51

These patients may present not only the hematologic picture of pernicious anemia but many of the other clinical features as well. Glossitis, icterus, paresthesias, and occasionally evidences of organic involvement of the spinal cord have been observed. The macrocytic anemia is apparently due to inadequate absorption of the antianemic or maturation factor from the intestines. The bone marrow presents a picture similar to that of pernicious anemia.

Treatment. The primary feature in the treatment of such cases is to relieve the obstruction and to get rid of the areas of stasis. The administration of folic acid or a potent liver extract leads to improvement of the anemia. This is of value in the preoperative treatment in some cases and in controlling the anemia of those patients in whom short-circuiting operations have been done.

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An infestation with the fish tapeworm will occasionally cause a macrocytic anemia which is indistinguishable from pernicious anemia in so far as the hematologic and clinical features are concerned. The incidence of this type of anemia is not high even among those known to be harboring the parasite. Infestation with the fish tapeworm is common in Japan and in

the region about the Baltic Sea; its incidence appears to be increasing in the United States in the region about the Great Lakes.

Stomatitis, glossitis, and paresthesias are frequent in this type of anemia, and organic neural involvement occasionally occurs so that the clinical picture of pernicious anemia is closely simulated.

The reason for the development of a macrocytic anemia is not clear although it may be because of improper absorption of the antianemic factor. The possibility of destruction or inhibition of the gastric intrinsic factor by the parasite or its toxins has been suggested.

The anemia improves after the worm has been expelled, but the administration of liver extract will improve or cure the anemia even though the worm has not been expelled. It has also been shown that folic acid is effective in this type of anemia.

Achrestic Anemia

A few cases of hyperchromic macrocytic anemia have been observed in which there is a hematologic and clinical picture similar to pernicious anemia except that the gastric secretion is normal. The bone marrow in such cases has shown a megaloblastic hyperplasia. No lesions have been demonstrated in the gastrointestinal tract, and extracts prepared from the liver of patients dying with this type of anemia have produced a hematopoietic response in patients with pernicious anemia.

It has been suggested that the anemia in these patients is due to a failure of the bone marrow to utilize the antianemic factor, but this explanation has been questioned. The response obtained with the administration of liver extract is poor or entirely absent.

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IRON DEFICIENCY ANEMIAS

A MICROCYTIC HYPOCHROMIC TYPE OF ANEMIA DEVELOPS WHEN AN ADEQUATE amount of iron is not available for hemoglobin formation. The cause for this deficiency in available iron varies from one case to another, and although there are obvious gaps in our knowledge of iron absorption, storage, and utilization, certain facts have been established which explain the pathogenesis of these anemias. Iron is known to be present in most, if not all, of the cells of the body. The total amount of iron in a normal adult is approximately 4.5 to 5 Gm. This is present in three forms. The iron in the blood hemoglobin constitutes the largest portion, but the amount varies with the age, size, sex, and blood hemoglobin level of the individual. It is about 2.5 or 3 Gm. in the normal adult. The second form is the iron reserve found in the reticulo-endothelial tissues. This is available for hemoglobin formation, but its amount cannot be estimated with any degree of accuracy and undoubtedly varies widely from one individual to another. It is stored in the liver, spleen, bone marrow, and elsewhere in the reticulo-endothelial tissues and has been estimated to amount to about 1.5 Gm. A third form of iron occurs in the nuclei and cytoplasm of all cells. This iron is not available for hemoglobin formation.

Most of the iron is absorbed in the upper portion of the small intestine and is excreted almost entirely in the lower bowel. The complete picture of iron metabolism is obscured by the fact that there is no available method of separating that part of the iron which has passed through the gastrointestinal tract unabsorbed and unchanged from that which has been absorbed, utilized, and excreted into the bowel. The urinary excretion of iron is quite constant in each individual, but the amount eliminated by this route is not sufficient to play a part in the development of an iron deficiency.

The acidity of the gastric juice plays an important role in the absorption of iron from the gastrointestinal tract. Achlorhydria appears to be an im-

portant factor in producing an iron deficiency. Experimental work has shown that achlorhydria definitely interferes with the absorption of dietary iron, and the frequency with which it is associated with hypochromic anemia has been repeatedly emphasized in clinical reports.

Opinions differ as to the amount of iron which is required each day by a normal individual, the estimates ranging from 5 to 15 (or more) mg. The available evidence suggests that the dietary iron intake in the adult should be in the neighborhood of 12 to 15 mg. per day to insure absorption of an adequate amount. Not all of the iron present in various foodstuffs is available for absorption and utilization. The iron requirements of children vary with their age. In infancy the amount needed each day is about 1 mg. per kilogram of body weight. This requirement declines with advancing years so that by the age of 4 or 5 years it has decreased to 0.5 or 0.6 mg. per kilogram of body weight. It is difficult to meet the need in an infant by dietary means except with the use of cereals fortified with iron. The average diet of older children contains an adequate amount.

Two of the most common factors which increase the iron requirement of an individual are (1) the iron lost in the form of hemoglobin by hemorrhage and (2) the amount needed in the formation of new iron-containing tissues. When blood is lost from the body, obviously iron is necessary for the regeneration of hemoglobin to replace it. If the hemorrhage occurs repeatedly over a long period of time, there may not be sufficient iron absorbed from the food to replace what is being used in the formation of new hemoglobin. In this way the available reserve supply of iron becomes depleted. The loss of blood during menstruation cannot be entirely disregarded in this connection, for although the average loss, about 50 cc. of blood or 20 mg. of iron per period, can be readily replaced, this does not hold true in cases of menorrhagia.

The iron necessary for the growth and development of iron-containing tissues and cells in the body is an important aspect of iron metabolism. It is important during pregnancy since the fetus at the time of birth contains in the neighborhood of 350 to 400 mg. of iron, all of which must be obtained from the mother. Supplying this amount of iron constitutes a severe drain on the maternal reserves, particularly when there are other complicating factors. An excessive demand for iron is also present during infancy and childhood when the rate of growth is rapid. A deficiency may develop at this time if the fetal reserve supply was inadequate at the time of birth or if this supply is not replenished by an adequate dietary intake. The need for large amounts

of iron continues during childhood and seems to be particularly great at the time of adolescence and puberty when, it has been estimated, the need is nearly as great as during a pregnancy.

Since the iron deficiency may be brought about in a number of ways, it is obvious that a wide variety of conditions may cause a hypochromic anemia of this type. It may be caused by gastrointestinal disorders, such as achlorhydria or a persistent diarrhea, which interfere with absorption of iron, by inadequate diets, by chronic loss of blood, by the excessive demand for iron during the period of life when growth is rapid, or by the fetal demands for iron during pregnancy. As these factors may act alone or in various combinations, the appearance of an anemia of this type may require extensive study to determine the true cause.

BLOOD PICTURE IN IRON DEFICIENCY ANEMIAS

All cases of iron deficiency anemia present a similar hematologic picture. The following description is applicable to all the clinical types. The anemia is characterized by a reduction in the blood hemoglobin level which is proportionately greater than the reduction in the number of erythrocytes. A low color index results, frequently as low as 0.5, but seldom below this. This indicates that each red cell contains less than the normal amount of hemoglobin. The mean corpuscular hemoglobin concentration is found to be below normal. The hematocrit reading is low, and this reduction in the volume of the packed erythrocytes is also proportionately greater than the reduction in the number of erythrocytes. A low volume index results, and the mean corpuscular volume is found to be decreased—an indication that the average size of the erythrocytes is less than normal.

These features classify the anemia as hypochromic and microcytic in type. They are emphasized by examination of the smear (Fig. 16), on which the erythrocytes are found to be pale and hypochromic. There are many ring, pessary, or ghost cells in which the small amount of hemoglobin present in the cell is concentrated at the periphery, leaving an extremely large central pale area (Fig. 17). The cells are smaller than normal and few macrocytes are found although there are some variations in the size and shape of the erythrocytes. A Price-Jones curve (Fig. 18) demonstrates this microcytosis. Polychromasia is not commonly encountered and normoblasts are rare, usually appearing only as a response to a recent profuse hemorrhage. The reticulocytes are not greatly increased in number. Aside from the alterations in the red cells and hemoglobin, there is little of significance to be found on

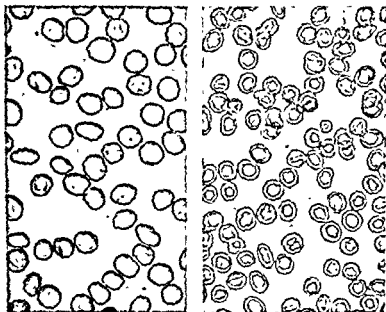


FIG. 16 Photomicrograph of erythrocytes. On the left are normal cells. On the right are pale, hypochromic cells from a case of iron deficiency anemia.

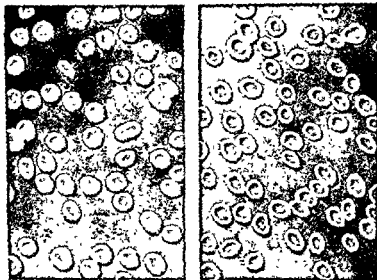


FIG. 17. Bas-relief photomicrographs of erythrocytes. On the left are normal cells. On the right are cells from a case of iron deficiency anemia.

examination of the blood smear. The total leukocyte count is normal, and there is a normal distribution of the cells on differential count. A moderate leukopenia with a relative lymphocytosis has been found in a few instances. The platelets are unaltered except when the picture is complicated by a recent hemorrhage. This may lead to an increase in the numbers of both platelets and leukocytes. There is no significant alteration in the fragility of the erythrocytes and no jaundice or elevation of the serum bilirubin.

The bone marrow in iron deficiency anemia shows a hyperplasia in which the normoblasts are increased in number and predominate in the smear. The

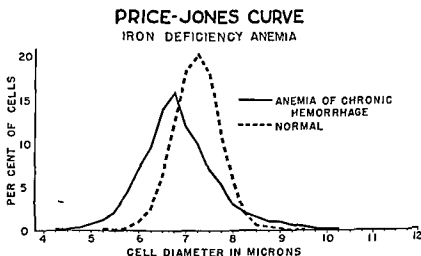


FIG. 18. The curve shows that a majority of the cells in iron deficiency anemia have a diameter which is less than the normal, the peak of the curve falling at 6.5 microns.

picture is in sharp contrast to the marrow in pernicious anemia in which megaloblasts predominate. The findings are those of active erythropoiesis and mitotic figures are common. As the blood returns to normal under proper therapy the marrow likewise becomes normal.

CLINICAL TYPES OF IRON DEFICIENCY ANEMIAS

Hypochromic Anemia in Infants

The hypochromic anemia of infancy and childhood is not present at birth nor does it appear immediately thereafter. It is prone to develop during the first year of life, frequently making its appearance soon after the fourth month at a time when the child's growth is rapid and the demand for iron is particularly great. The blood hemoglobin and erythrocyte count of a

normal infant are high at birth but they decline rapidly. The downward trend continues to the point at which an anemia develops when there is a deficiency of iron. This is apt to become particularly severe in infants who are small at the time of birth and whose growth is unusually rapid. It is also prone to develop in premature infants in whom there was not sufficient time for the iron stores to become adequately filled by the time of birth. It is more frequent in the case of twins and multiple pregnancies to whom the maternal organism has been unable to supply an adequate iron reserve. The blood hemoglobin is characteristically normal during the early months of life in those infants born to a mother who has an iron deficiency type of anemia, but the probabilities of an anemia developing during the first year are far greater than in infants whose mother does not have such an anemia.

Anemia may also develop if the reserve supply of iron is not adequately replenished by dietary means. Inadequate replenishment is most frequently encountered in infants receiving cow's milk rather than breast milk. The iron content of the former is much lower, and iron retention from artificial feedings is far lower than from breast milk. An inadequate iron intake also results from withholding supplemental feedings for too long a time so that the infant is being maintained on a milk diet at a time when other foods such as vegetables, eggs, and cereals should be given. In other instances the iron intake is too low because the supplemental feedings are not of the right type.

The anemia in these infants is the result of inadequate iron stores, an inadequate iron intake, or a combination of these, during a time when there is an excessive demand for iron because of rapid growth. Parsons has given the following causes for the development of an iron deficiency in children:

1. *Deficient antenatal storage*
 - a Iron deficiency in the mother
 - b Deficient transference of iron to the fetus
 - c Prematurity of fetus and insufficient storage
 - d Twins, iron stores obtainable from the mother being insufficient for both children
2. *Deficient postnatal supply*
 - a Insufficient supply of iron in the breast milk, possibly from iron deficiency in mother's diet
 - b Artificial feeding with cow's milk, which gives an iron retention only one-fifth that of breast feeding
 - c Prolongation of milk feeding beyond the normal lactation period
3. *Deficiency of both antenatal storage and postnatal supply*

The clinical picture in these children is not particularly striking except for a variable degree of pallor and listlessness. They may appear well nourished, well developed, and otherwise healthy. There is no icterus. The

spleen is often palpable but there is no lymphadenopathy. The children are somewhat more susceptible to infections than are those with a normal blood hemoglobin.

Nutritional Iron Deficiency Anemia in Adults

A severe grade of hypochromic anemia is seldom encountered in an adult as a result of a low iron intake alone. Mild grades of anemia on this basis are not infrequent. A significant degree of anemia is not uncommon when a low iron intake is combined with other factors which interfere with absorption or utilization of the iron. The usual diet of the average adult contains from 10 to 20 mg. of iron per day. Even though all of this is not absorbed, it is sufficient for ordinary metabolic needs. The actual requirement for iron is considerably below the usual daily intake so that even with a restricted diet an actual deficiency develops very slowly, and anemia seldom progresses to a severe stage. The foods which contribute most of the iron to the diet are red meats and liver, green vegetables, carrots, egg yolk, and certain fruits such as apricots, peaches, dates, figs, raisins, and prunes. Smaller amounts of iron are found in many other fruits, berries, and vegetables.

Although the diet of a normal adult is seldom restricted to the point of producing a severe anemia, a mild degree of anemia from this cause is not infrequently encountered. This is particularly frequent in young women, usually appearing at an age when dietary fads and dislikes are particularly common and when the demand for iron is still high because of growth and development plus the added demand to compensate for that lost with the menstrual periods. Blood hemoglobin levels of from 9 to 11 Gm. per 100 cc. of blood are common in females of this age group. When such a mild anemia is present, the manifestations are not striking. Loss of pep, fatigability, and weakness are the usual complaints. We have found mild grades of anemia in many persons who have had no specific complaints although after their blood hemoglobin has been brought to a normal level by means of iron therapy, they feel much better than they did previously. Their weakness and fatigability were so mild and had come on so gradually that the patients had not recognized their presence.

The anemia may become severe when a low iron intake is associated with some factor that interferes with iron absorption. This may occur with an achlorhydria which decreases the absorption of iron or when large amounts of alkaline powders are given in the treatment of peptic ulcer. A persistent diarrhea, as with amebic and bacillary dysentery or chronic ulcera-

tive colitis, may also lessen the absorption of iron. Other gastrointestinal lesions which lead to poor absorption may act in a similar manner and may lead to a severe hypochromic anemia.

Chlorosis

Chlorosis, or "green sickness," is a form of hypochromic anemia which occurs in adolescent girls, usually between the ages of 14 and 20. The term *chlorosis* as well as the popular name "green sickness" was applied to the disease because of an apparent greenish tint to the skin beneath the eyes and the chin. Some observers who were accustomed to seeing many patients with chlorosis have cast doubt on the actual presence of a greenish discoloration.

Many authors have alluded to chlorosis under one name or another since it was first described by Johannes Lange in 1510. During the succeeding three centuries it was apparently a prevalent and conspicuous disease, so conspicuous in fact that it was portrayed on canvas by many famous artists of the seventeenth century. Since the end of the nineteenth century its incidence has rapidly decreased, until at the present time it is extremely rare in its classical form. Many cases of anemia which are now recognized as being due to nephritis, tuberculosis, chronic hemorrhage, or dietary deficiencies were undoubtedly called chlorosis in the past, but this greater accuracy in diagnosis does not seem to entirely explain the decline in incidence. It is possible that improved dietary habits and better living conditions may be partially responsible for its disappearance.

The cause of chlorosis has never been adequately determined. It seems probable that a low iron intake plus an excessive demand for iron during adolescence and the onset of menses were the etiologic factors. Many girls with mild grades of hypochromic anemia are encountered today in the chlorotic age group although few of the classical symptoms are present and the anemia is usually not severe. Hematologic studies on girls entering nurses training at the University Hospitals have shown a mild anemia to be prevalent. It is much more frequent in females than among males of the same age group. These data show that though chlorosis in its classical form has largely disappeared, a similar but mild grade of anemia in females of this age group is still a common finding.

The symptoms of chlorosis are those of any anemia: weakness, loss of pep, fatigability, and slight palpitation. These are present in varying degrees depending upon the severity of the anemia. The pallor is also variable. A clear pearly white pallor will be noted in a blond patient. There will be a

yellowish tint to the skin of a brunette because the normal skin pigment shows more prominently. The yellowish tint is not apparent in the scleras. Dyspepsia and perverted appetites have been described as frequent symptoms of chlorosis, and menstrual irregularities are common. Examination reveals little aside from the pallor. There is no icterus, the serum bilirubin is not elevated, there is no lymphadenopathy, and the spleen is seldom enlarged. Achlorhydria is rare in this disease, gastric hyperacidity being a more common finding.

The blood picture is that of a hypochromic anemia. An indication of the prevalence of this disease in the past is shown by the use of the term *chlorotic type of anemia* to describe what is now called a hypochromic anemia.

Anemia of Chronic Hemorrhage

The hypochromic anemia caused by chronic blood loss is the most common form of iron deficiency anemia. It may be encountered at any age and from a wide variety of lesions but is particularly frequent with bleeding hemorrhoids, a slowly bleeding peptic ulcer, and menorrhagia. Hemorrhage is an important factor in the production of the anemia associated with carcinoma of the stomach and colon, ulcerative colitis, hookworm infestation, and a great many other diseases.

The hemoglobin which is lost with a single hemorrhage, even though it is large, can be replaced with ease by drawing on the reserve stores of iron in the body. When this demand for iron is repeated at frequent intervals over a long period of time, the available iron becomes exhausted, and the body stores cannot be replenished as rapidly as they are being used. The resultant iron deficiency leads to a slower formation of hemoglobin, and the typical hypochromic microcytic type of anemia results. Since there is no interference with the production of erythrocytes themselves, but rather with the production of hemoglobin, each cell contains less than its normal complement of hemoglobin. The individual cells are consequently small and pale.

Chronic hemorrhage is a factor in the production of a large percentage of the severe forms of iron deficiency anemia. Rhoads has stated that a microcytic anemia is seldom encountered in the absence of hemorrhage. For this reason it is necessary to institute a painstaking search for evidence of blood loss whenever a hypochromic microcytic anemia is encountered.

Carcinoma of the ascending portion of the colon is prone to cause a severe hypochromic anemia because of the constant but unrecognized loss of blood,

in this location the growth may cause few bowel symptoms. This condition must always be considered in the diagnosis of an otherwise unexplained hypochromic anemia in an older patient. The anemia is commonly the presenting symptom of the lesion.

Hypochromic Anemia of Pregnancy

A moderate reduction in the amount of hemoglobin per unit volume of blood occurs as a normal phenomenon in pregnancy. This is the result of hydremia, an increase in the total plasma volume. Hemoglobin values as low as 10 or 11 Gm. per 100 cc. of blood with a corresponding reduction in the number of erythrocytes may occur as a result of hydremia and need not be considered as pathologic after the fourth or fifth month of pregnancy. Readings below 10 Gm. of hemoglobin are probably never physiologic. When such a low reading is associated with a low mean corpuscular hemoglobin content or a low color index, it is indicative of an iron deficiency anemia. This type of anemia of pregnancy is more frequent than the macrocytic and hyperchromic form. Strauss and Castle studied 38 consecutive patients having an anemia of pregnancy and found 30 of them to be of the hypochromic type. In the University Hospitals there were 4 cases of hyperchromic anemia as compared to 406 cases of the hypochromic type in 11,370 consecutive pregnant women.

Several factors may contribute to hypochromic anemia of pregnancy. There is an excessive demand for iron by the fetus since all the iron for blood hemoglobin, the tissues, and the reserve supply must be obtained from the mother. This excessive demand depletes the maternal stores just as would a corresponding loss of iron by hemorrhage, and the dietary intake of iron during the last trimester of pregnancy is ordinarily insufficient to meet the demand. Since relatively few pregnant women develop this type of anemia, it is obvious that other factors must contribute. A diet which is deficient in iron has been taken by many of these patients either because they are financially unable to purchase the necessary foods or because a perverted or peculiar appetite has prevented their use. Persistent nausea and vomiting seriously interfere with the absorption of iron from the gastrointestinal tract in other patients. An achlorhydria or hypochlorhydria is an almost constant feature in the hypochromic anemia of pregnancy and, as has been shown, this interferes with the absorption of dietary iron. A low gastric acidity is therefore one of the most important etiologic factors. Repeated and frequent pregnancies, particularly when complicated by low iron absorption, are apt to lead to especially severe grades of anemia.

The clinical features are those of any anemia. Weakness, lassitude, and fatigability are the predominating symptoms in a majority of the cases. Palpitation and shortness of breath on exertion are likely to appear in the more severe forms. In many instances the symptoms are entirely masked by those of the pregnancy so that the anemia is detected only by the routine blood examination.

Varying degrees of pallor may be noted, but there is no jaundice. Although atrophy of the tongue is occasionally encountered, glossitis with pain and burning is infrequent. The finger nails may be thin and brittle, but spoon-shaped nails, such as are found in idiopathic hypochromic anemia, are not common. The spleen is occasionally enlarged to a slight degree but subsides to its normal size with improvement of the anemia. Achlorhydria or hypochlorhydria is an almost constant feature. In many instances a subsequent pregnancy occurs before the patient has fully recovered from her anemia. This results in an even more severe anemia than was present during the preceding pregnancy.

Idiopathic Hypochromic Anemia

Idiopathic hypochromic anemia is another type of iron deficiency anemia in which there are multiple etiologic factors. It occurs most frequently in middle-aged women with low or absent free hydrochloric acid in their gastric contents. The clinical and hematologic features correspond to those of the other types of iron deficiency anemia, but certain variations have tended to set this form apart as a separate symptom complex. The exact incidence is unknown as some confusion exists concerning the exact criteria for inclusion of an anemia in this category. It is less common than the anemia of chronic hemorrhage.

The onset is insidious. It is difficult to determine the length of time the disease has been present although a history of five or more years of weakness and ill health is common. Sometimes the patient dates the onset to a severe infection, operation, or other illness. In other instances the onset may be traced to a preceding anemia of pregnancy from which the patient did not fully recover. The symptoms are usually vague and consist primarily of weakness, fatigability, and loss of strength together with palpitation, shortness of breath, and nervousness. Patients seem to become adjusted to their state of chronic ill health because the onset and progress are so gradual. The shortness of breath, palpitation, and other symptoms referable to the cardiovascular system are similar to those of other severe anemias.

Vague symptoms referable to the gastrointestinal tract are common,

anorexia, nausea, constipation, dyspepsia, and eructations of gas are among the most frequent. Vomiting and diarrhea occasionally appear and may lead to such a careful selection of food that the diet ultimately becomes inadequate. Soreness and burning of the mouth and tongue are occasionally encountered, but the glossitis is seldom as pronounced as in pernicious anemia.

Dysphagia is a particularly prominent feature of this type of anemia. It was present in 6 of the 11 cases which we studied in detail. The difficulty in swallowing was so severe in two of the patients that they were referred to this hospital with a diagnosis of carcinoma of the esophagus. In the absence of a roentgenologic examination or esophagoscopy, it is easy to see how such a diagnosis might be made in an elderly patient with a severe hypochromic anemia who has great difficulty in swallowing. Solid foods ordinarily cause greater difficulty than liquid foods, and the dysphagia is frequently more severe when the patient is tired. Examination by means of an esophagoscope has shown no constant pathologic picture although some atrophy of the esophageal mucosa may be present. The dysphagia is probably due to muscle spasm and entirely disappears with iron therapy. When dysphagia accompanies this type of anemia it is commonly called the "Plummer-Vinson syndrome."

Numbness and tingling of the extremities have been noted rather frequently but are less severe than when encountered in pernicious anemia. They are not associated with evidence of organic lesions in the spinal cord.

Menorrhagia is a feature in this type of anemia although it may not have been noted by the patient. Actual measurement of the menstrual blood loss shows it to be excessive in many of the patients, and iron balance studies show that the iron which is lost exceeds the amount which is being retained from the diet. It is probable that an excessive menstrual blood loss is an important etiologic factor in most of these patients.

Examination reveals a pallor which, by the time the patient consults a physician, is usually quite marked. It is a clear pallor which is not complicated by icterus although in brunettes a yellowish tint may be apparent in the skin. This is distinguishable from true icterus by the fact that the scleras are pearly white and the serum bilirubin is normal or low. Most patients maintain a good state of nutrition and do not appear emaciated. Weight is normal although the patients seem listless, apathetic, and fatigued. The hair is frequently of fine texture, dry, and gray. Atrophy of the papillae of the tongue was present in 64 per cent of Wintrobe's cases. This may be apparent only at the edges of the tongue or it may be as striking and severe as that found in pernicious anemia (Fig. 19).

Alterations in the finger nails are frequently encountered, the most com-

mon manifestation being nails which are thin, brittle, and easily broken and which have longitudinal ridges. In the more extreme cases there is a flattening or actual concavity of the nails as is shown in Figure 20. This is spoken



FIG. 19. Atrophic glossitis in a patient with idiopathic hypochromic anemia

of as "spoon-shaped" nails or koilonychia. Such changes are not specific for idiopathic hypochromic anemia as they have been observed in males with an anemia due to a bleeding peptic ulcer. The nails may return to a normal state when the anemia is controlled.

The spleen is enlarged in about 50 per cent of the cases, but the size is never excessive. An increased area of splenic dullness may be noted on percussion, or the organ may be palpable one or two centimeters below the costal margin. It will usually recede to normal when the anemia has been controlled. The liver is seldom enlarged and there is no lymphadenopathy. There may be a slight degree of cardiac dilatation, and a systolic hernic murmur is common. A venous hum will occasionally be heard over the veins of the neck.

Gastric analysis reveals an achlorhydria in a large percentage of the patients and a hypochlorhydria in many of the others. In only 8.5 per cent of the patients was the gastric acidity normal (Wintrobe). Although free hydrochloric acid is low or absent, there is not an achylia as is found in pernicious anemia. Castle has demonstrated that the gastric contents of these patients contain the "intrinsic factor."

There is no evidence of increased blood destruction, and the serum bilirubin is normal. The reticulocytes may be slightly increased in number. Sternal puncture shows hyperplasia of the marrow with erythrocytes of the normoblastic stage predominating. There are no characteristic changes in the leukocyte count. The anemia is a microcytic hypochromic type as is found in other iron deficiency anemias.

Etiology

The principal factors concerned in the development of this type of anemia have been mentioned, i.e., menorrhagia and achlorhydria. The loss of an

excessive amount of blood with the menstrual periods is an important contributory factor as was demonstrated by actual measurements. The lowered gastric acidity interferes with the absorption of iron so that replacement of the iron lost with the menorrhagia is difficult. In a few cases there is a history of a diet of low iron content. The onset of the illness in some cases can be traced to a pregnancy or repeated pregnancies at relatively short intervals so that the disease represents a hypochromic anemia of pregnancy in which the hemoglobin deficit has never been replaced. We have not been able to find any evidence of a defect in internal iron metabolism or utilization.



FIG 20 Koilonychia, an abnormal concavity of the finger nails, in a patient with idiopathic hypochromic anemia

Examples of this type of hypochromic anemia and of pernicious anemia have been found in the same family. This high incidence of idiopathic hypochromic anemia in "pernicious anemia families" is undoubtedly due to familial achlorhydria. Cases of idiopathic hypochromic anemia which have terminated as typical pernicious anemia have been reported.

Diagnosis

The diagnosis rests on the foregoing clinical and hematologic features. An extremely careful search must be made for evidences of chronic hemorrhage in all patients, particularly for evidences of menorrhagia or chronic bleeding

from the gastrointestinal tract. Banti's syndrome in the early stage may present a somewhat similar picture, but there is usually a large spleen, leukopenia, and, in the late stages, evidences of portal obstruction and liver insufficiency. When glossitis and stomatitis are present, a deficiency in vitamin B must be considered. Aplastic anemia can be excluded by the platelet and leukocyte counts and by examination of the bone marrow. Pernicious anemia is excluded by its characteristic macrocytic hyperchromic blood picture.

The prognosis is good if the excessive blood loss, when present, can be controlled. The response to adequate amounts of iron is striking, and the blood picture may be brought to normal within a short time.

TREATMENT OF IRON DEFICIENCY ANEMIAS

The essential feature in the treatment of iron deficiency anemias is the administration of adequate amounts of an inorganic iron salt. The effectiveness of iron in the treatment of certain types of anemia has long been recognized, but for a time it was in disrepute because of the use of complex organic compounds or too small amounts of inorganic iron. Its routine and perfunctory use in all types of secondary anemia has been superseded by the more rational administration of adequate amounts of iron to properly selected cases. This therapeutic renaissance has been due in part to a better understanding of the pathogenesis of the anemias as well as to the recognition that large amounts of iron are advisable. The enthusiasm for massive doses of iron has perhaps resulted in the administration of excessive amounts to some patients but has led to excellent therapeutic results.

Numerous iron salts are effective in the treatment of iron deficiency anemias. Those which are presented here were selected only because they are the ones most commonly used and because they serve as examples of the various types of preparations. The average daily dose is that which will produce a maximal response in a majority of adult patients. It is realized that not all patients will show the same response to identical amounts of an iron salt and that some patients will respond satisfactorily to smaller amounts than those listed. Certain refractory cases may require even larger amounts.

Preparation	Daily Dose	Metallic Iron Content
Iron and ammonium citrates	6 Gm.	1000 mg
Reduced iron (<i>ferrum reductum</i>)	3 Gm.	2800 mg
Ferrous carbonate	4 Gm.	360 mg.
Ferrous sulfate	2 Gm.	400 mg.

Comparison of these iron preparations on a basis of their metallic iron content shows that the ferrous are more effective than the ferric salts. It has been demonstrated that ferrous salts are more readily absorbed from the gastrointestinal tract than ferric salts, and it has been suggested that iron may be absorbed only in the ferrous state. After absorption the iron is transported by the blood plasma. Moore has demonstrated that ferrous salts produce a greater and more rapid increase in the serum iron than do ferric salts. Because of the greater absorbability of ferrous salts, they can be given in smaller amounts and still produce an equally great response in hemoglobin regeneration. When the number of grams of iron utilized in hemoglobin formation is ascertained, it is found that there is no significant difference in the utilization of the two types of preparations. The difference between the ferrous and the ferric salts seems to lie in their absorbability rather than in their subsequent metabolism and utilization.

The response to adequate amounts of iron in properly selected cases is striking. There is first an increase in the number of reticulocytes which begins a few days after the administration of iron is started, but the reticulocyte response is slower and does not reach as high a peak as is true with liver extract in pernicious anemia (Fig. 21). The height of the response is inversely proportional to the hemoglobin level at the onset of treatment so that a higher reticulocyte count will occur in the more severe anemias. Following the reticulocyte rise there is an increase in the hemoglobin level and the erythrocyte count, but iron has a greater effect on the formation of hemoglobin than on the erythrocytes. The rapidity and extent of the hemoglobin increase depend upon the severity of the anemia, being more rapid and greater when the pretreatment hemoglobin level is lower. The increase in the blood hemoglobin is the best single index as to the adequacy of treatment.

Patients with idiopathic hypochromic anemia will, as a rule, require somewhat larger amounts of iron than patients with other types of iron deficiency anemias, and the dosage already listed applies to that type of case. In instances in which the anemia is due to chronic blood loss alone a smaller amount of the iron salt, one-half or less of the listed dosage, may give a maximal response (Fig. 22). The effectiveness of iron therapy will be decreased in the presence of nephritis, nitrogen retention, infections, hypothyroidism, vitamin deficiencies, or persistent chronic blood loss.

Iron and ammonium citrates are usually administered in the form of 0.5 Gm (7½ gram) capsules but may be used in a 25 or 50% aqueous solu-

tion in a suitable vehicle. This is one of the best of the complex ferric compounds. It is soluble, and when taken in a liquid form precautions must be observed to prevent its coming in contact with the teeth. It is best taken through a glass tube. Reduced iron, an insoluble preparation, is conveniently administered in capsules containing 0.5 Gm. ($7\frac{1}{2}$ grains) of the drug. Ferrous carbonate is prepared in the form of pills containing from 0.3 to 0.6 Gm

HYPOCHROMIC ANEMIA

RETICULOCYTE RESPONSE TO IRON THERAPY

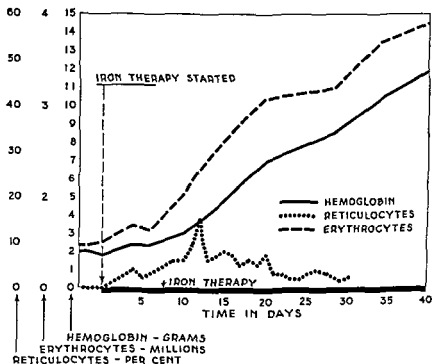


FIG. 21. Showing the response of the reticulocytes, erythrocytes, and hemoglobin to iron therapy in a case of iron deficiency anemia.

(5 to 10 grains) which are coated to prevent oxidation of the drug before administration as well as to prevent gastric irritation. Ferrous sulfate is also dispensed as coated pills, usually containing from 0.3 to 0.5 Gm. (5 to $7\frac{1}{2}$ grains) of the salt.

Untoward symptoms from the administration of iron, usually in the form of gastric irritation or diarrhea, are not infrequently encountered. The nausea, vomiting, and diarrhea are more frequent when the soluble salts of iron are used. We have encountered the least trouble with reduced iron.

occasionally finding patients who could take this form when none of the other salts could be tolerated. Diarrhea may begin a few days after the administration of the drug is begun, last for several days, and then spontaneously subside even though the administration of iron is continued. Ferrous salts,

CHRONIC HEMORRHAGIC ANEMIA

EFFECT OF IRON AND AMMONIUM CITRATES 3 GRAMS DAILY

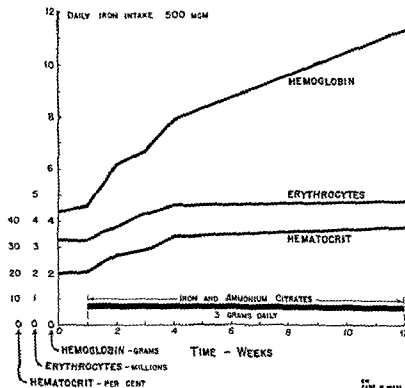


FIG. 22 The rate of hemoglobin increase in this patient with chronic hemorrhagic anemia is satisfactory even though the dosage of iron is only half that which is employed

because of their smaller dosage, may be well tolerated, but the forms such as the sulfate and chloride frequently are irritating. None iron salts should be given on an empty stomach, they are best administered either with or immediately following a meal. Iron has been found to be effective when given at frequent intervals rather than in a single dose.

that dividing the daily dose into three parts and giving it with the meals not only diminishes the gastrointestinal irritation but increases its effectiveness on hemoglobin formation.

Although achlorhydria interferes with the absorption of iron from the dietary iron intake, there is adequate absorption when large amounts of medicinal iron are given even though free hydrochloric acid is absent from the gastric contents. The administration of hydrochloric acid in conjunction with the iron salt does not appreciably increase the rate of hemoglobin formation, consequently its use is not essential. Dilute hydrochloric acid may aid in relieving the gastrointestinal symptoms due to achlorhydria.

Intramuscular injections of iron cannot exceed 16 to 32 mg. per day because of the toxic reactions. In this small dosage the effect on hemoglobin formation is not great. This route of administration is not advisable or effective. We have encountered patients in whom there was no hemoglobin response to intramuscular iron even when given to the point of producing mild toxic reactions. But when the drug was given orally to the same patients, a prompt and satisfactory hemoglobin response ensued. Large doses of iron in a colloidal state have been administered intravenously to patients by Moore and his co-workers but the toxic reactions were so severe that they concluded that this route of administration was contraindicated. There was, however, an exceedingly high reticulocyte response, a rapid rate of hemoglobin regeneration, and a high percentage of utilization of the administered iron.

Copper is necessary as a catalytic agent in the synthesis of hemoglobin even though it does not enter into the structure of the hemoglobin molecule. In the adult, however, there is sufficient copper in the food and as a contaminant of the iron salts so that additional copper is not necessary. The administration of both copper and iron to a group of 20 adult patients did not produce any more rapid hemoglobin response than did the administration of iron alone.

The epoch-making experiments of Whipple and his associates on the effect of various foodstuffs on hemoglobin formation in dogs have shown the beneficial effects of liver and liver extracts. The liver fraction which is precipitated by 70% alcohol and an aqueous extract of liver were found to be effective in the treatment of chronic hemorrhagic anemia. That subfraction of liver which is effective in the treatment of pernicious anemia is of no value in iron deficiency anemia. Iron produces a greater response than does liver or liver extract so that the administration of these, either alone or in combination with an iron salt, is not necessary. A few cases of iron deficiency anemia are complicated by other nutritional deficiencies. In these patients the addition of liver or a liver fraction may hasten hemoglobin formation.

Combinations of iron and various vitamins are commonly employed in the treatment of these anemias, but unless there is evidence of a vitamin deficiency, they have no advantage over a simple iron salt. Such combinations are not to be recommended since a majority of them contain too little iron to be effective and are an unnecessary expense to the patient.

A maintenance dose of iron is required in some patients for an indefinite period after the hemoglobin has been raised to a normal level. This is especially true in idiopathic hypochromic anemia. Iron may be given continu-

RETENTION AND UTILIZATION OF IRON

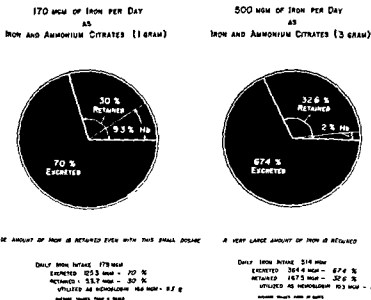


FIG. 23. Showing the amount and percentage of iron retained and utilized with the administration of iron and ammonium citrates (Fowler and Barer, *Ann. Int. Med.*)

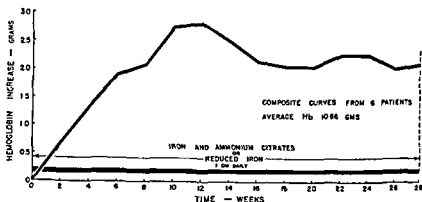
ously in small doses or intermittently in larger amounts. The maintenance dose may be considerably smaller than that used in raising the hemoglobin level to normal.

It is useless to speculate on the minimal effective dose of iron since this is so variable and, from an economic standpoint, the iron salts are so inexpensive. Iron balance studies have shown, however, that the amount of iron retained by the body from the oral administration of the drug is far greater than the amount utilized in hemoglobin formation. Even with small doses, such as 1 Gm. of iron and ammonium citrates per day, an excess of iron was retained. When larger amounts of the same preparation were administered,

3 Gm. of iron and ammonium citrates per day, the amount of iron retained was still larger although the percentage of the administered dose which was

EFFECT OF IRON ON MILD ANEMIA

THE HEMOGLOBIN REMAINS ELEVATED ABOVE THE PRETREATMENT LEVEL



EFFECT OF IRON ON LOW NORMAL HEMOGLOBIN

THE HEMOGLOBIN RETURNS TO THE PRETREATMENT LEVEL

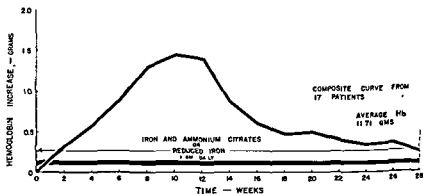


FIG. 24. Effect of iron therapy on patients with a mild hypochromic anemia and on individuals having a low normal hemoglobin level (Fowler and Bate, *Am J. M. Sc*)

used in hemoglobin formation was lowered. These data are shown in Figure 23.

Small amounts of various iron preparations, such as 1 Gm. of iron and ammonium citrates, 1 Gm. of reduced iron, and 0.36 Gm of ferrous sulfate per day, produce a satisfactory hemoglobin response in many patients. This

is especially true in the anemia due to chronic hemorrhage. The response to small amounts of iron does not mean that such small doses should be employed routinely.

Iron medication in hypochromic anemia is usually considered a form of replacement therapy, but the possibility that iron has a stimulating effect on the bone marrow and on hemoglobin formation cannot be entirely ignored. When it is administered to a patient with a moderate grade of anemia, the maximum increase in the blood hemoglobin level is reached at the end of eight to ten weeks. Subsequently there is a gradual drop in the hemoglobin

EFFECT OF IRON ON ACUTE HEMORRHAGIC ANEMIA

THE RECOVERY PERIOD, FOLLOWING REMOVAL OF 500 cc OF BLOOD, IS SHORTENED BY THE ADMINISTRATION OF IRON.

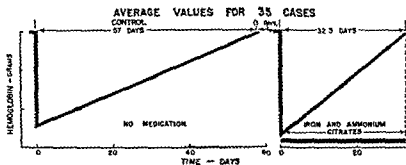


FIG. 15. Showing the effect of iron on hemoglobin regeneration in acute hemorrhagic anemia (blood donors)

so that it ultimately levels off at a point above the pretreatment level but below the maximum level attained. This type of response was obtained when iron was given continuously as well as when it was administered for sixty days and then discontinued. When iron was administered to a group of individuals having a low normal hemoglobin level, rather than an actual anemia, a similar type of response was observed (Fig. 24). The maximal response was obtained at the end of eight to ten weeks, but even though the administration of iron was continued, the hemoglobin dropped to the pretreatment level with no persistent increase.

The administration of iron will hasten the regeneration of hemoglobin in patients with an acute hemorrhagic anemia and in donors who have given

blood for a transfusion (Fig. 25). There is no actual deficiency in these cases but iron can apparently be made more readily available for the synthesis of hemoglobin. The iron content of the blood serum increases rapidly after the oral administration of an iron salt, and the experimental work with radioactive iron indicates that it soon appears in hemoglobin and erythrocytes.

The amount of iron administered to infants is much less than that given to adults. It is necessary to employ a soluble preparation and one which is not irritating to the gastrointestinal tract. The most widely used preparation is iron and ammonium citrates in a 1% solution—4 cc. daily up to the age of 6 months and 8 cc. daily from 6 to 12 months of age. Iron and ammonium citrates may also be given to older children in a 10% solution—2 cc. per Kg. of body weight per day. Iron salts which must be administered in the form of pills or capsules are not suitable for infants although they may be given to older children. If copper has any place in the treatment of anemia, it is in the hypochromic anemia of infants. An occasional case apparently improves more rapidly with additional copper. This may be given as a 0.5% solution of copper sulfate, the dose being 1 cc. per Kg. of body weight. It is best given in milk.

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APLASTIC ANEMIA

APLASTIC ANEMIA IS CHARACTERIZED NOT ONLY BY A REDUCTION IN THE NUMBER of erythrocytes and the amount of hemoglobin in the peripheral blood but also by a reduced number of leukocytes and platelets. All of the blood elements produced in the bone marrow are affected. It is a progressive anemia with little or no evidence of erythrocytic regeneration. The disease is recognized in idiopathic and secondary forms which are identical in their clinical and hematologic features, differing only in that some chemical, toxic, or infectious basis can be detected in the secondary form. It is possible that all cases are actually of the secondary type and we have simply failed to recognize the etiologic agent in those now called idiopathic. It is also possible that all the patients have a congenital but latent inadequacy of the bone marrow which is not apparent under ordinary circumstances but becomes evident when these individuals are exposed to certain chemicals or toxins that might produce no changes whatsoever in a normal person. If the latter concept is true, the apparent causative agent is merely a "trigger mechanism," which brings to light a latent defect in hematopoiesis. It is impossible at the present time to say which, if either, of these concepts is correct.

Etiology

Aplastic anemia in its secondary form has followed exposure to numerous drugs and chemicals, the most frequent being benzol (benzene) or one of its derivatives. This chemical is employed in many industries such as in the manufacture of rubber products, nitrocellulose, and other explosives, as a solvent for enamels, lacquers, and other coatings, and as a basis for many drugs, dyes, and chemicals. Reports from industrial plants indicate that benzol poisoning in a mild form is quite common and that inhalation of the fumes is the most important way of entry. Only a few of those exposed to the fumes develop aplastic anemia. Examination of workers who have been exposed shows that some of them have a reduction in the leukocyte count,

the erythrocyte count, or both, even though there have been no subjective manifestations of poisoning. There is apparently a great individual variation in the susceptibility to benzol poisoning, symptoms appearing in one person after slight exposure and not in others even after long exposure. The symptoms may appear soon after exposure or they may not become apparent until long after the person has been removed from contact with benzol.

The administration of organic arsenical preparations is occasionally complicated by the development of aplastic anemia. The incidence of such intoxication is extremely low when the number of cases developing aplastic anemia is compared to the great number of individuals receiving these drugs in antisyphilitic therapy. There is no relationship between the size of the dose and the appearance of anemia so that the individual susceptibility must be the deciding factor as to whether or not such an intoxication develops. It is of significance to note that aplastic anemia does not follow the use of inorganic arsenic but occurs only with the use of organic preparations which contain the benzene ring. Many other drugs, dyes, and chemicals have been suggested as causative agents, but in many instances they either have been condemned on insufficient evidence or have a benzene ring in their chemical structure.

Exposure to radioactive substances and roentgen rays will produce aplastic anemia. The incidence of bone marrow involvement was high until proper protection was given to workers exposed to these rays. The recent studies on the effects of atomic bomb explosions show that aplastic anemia is one of the serious sequelae, although evidences of regeneration appear relatively early in the bone marrow. Gold salts, dinitrophenol, bismuth, mercury trinitrotoluene, sulfonamides, tridione, and other drugs and chemicals have also been reported to have caused this type of anemia.

Aplastic anemia has been said to follow overwhelming infections or sepsis, but in a majority of such cases the infection was probably secondary, gaining its foothold because of the associated leukopenia and the lack of adequate defense against the organisms. The former belief that aplastic anemia might be a terminal event in pernicious anemia or in chronic hemorrhagic anemia has been discarded.

Pathology

The term *aplastic anemia* implies that there is aplasia or hypoplasia of the bone marrow of such a degree that erythrocytes cannot be supplied in adequate numbers to maintain their normal level in the blood stream. In the typical case that portion of the marrow which is concerned with hematopoie-

sis becomes pale and yellowish in color because fat tissue has replaced the cellular elements. In this aplastic marrow there is a reduction in the number of erythrocytes and erythropoietic elements and also a reduced number of myeloid cells and megakaryocytes (Fig. 26). The aplasia is not uniform. Small areas of hyperplasia containing many immature erythrocytes are found

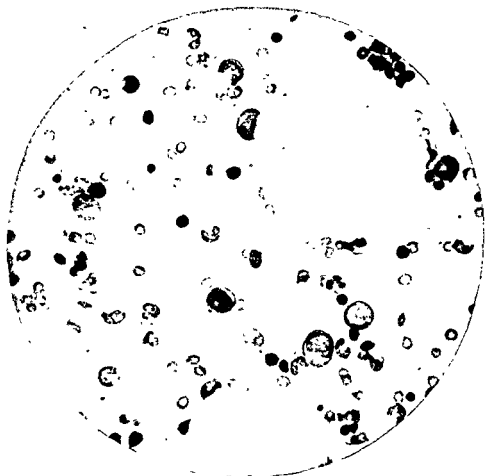


FIG. 26. Bone marrow smear in aplastic anemia. There is a sparsity of both erythrocytes and immature myeloid cells (Hal Downey, Handbook of Hematology Paul B Hoeber, Inc.)

interspersed between the fat cells. The presence of these scattered hyperplastic areas accounts for the few reticulocytes that appear in the blood stream. Since the involvement is patchy, it is obvious that a specimen of marrow obtained by sternal puncture may not give a picture which is representative of the condition of the entire marrow. In a series of cases having the characteristic clinical and hematologic findings of aplastic anemia we

found aplasia of the bone marrow, either diffuse or patchy, in all autopsied cases. Reports from other observers have shown that aplasia of the bone marrow is not a constant feature. Normal or hyperplastic bone marrow has been found in many patients who present all the clinical features of aplastic anemia.

Although aplastic anemia must be considered the result of bone marrow insufficiency, but this insufficiency may be on either a functional or an anatomic basis, and the disease represents a clinical rather than a pathologic entity.

The effects of chronic benzol poisoning on the bone marrow have been found to be as variable as those encountered in idiopathic aplastic anemia. Hypoplasia, normal cellularity, and marked hyperplasia have all been encountered after exposure to benzol. The same variable pathologic changes have been produced by benzol poisoning in experimental animals. Mallory believes that the intensity and duration of exposure to benzene influence the pathologic picture—that hyperplasia occurs only after short exposure, whereas hypoplasia is more frequent in males whereas hypoplasia was more common in females. The changes in peripheral blood are variable in benzene poisoning. The erythrocyte and leukocyte counts may be either elevated or depressed. A blood picture suggestive of myelogenous leukemia has been encountered in several instances.

The nitrogen mustard gases developed for chemical warfare produce changes in the bone marrow, hematopoiesis being affected either by the inhalation of a large amount of gas or by repeated exposures to low concentrations. After exposure to the gas in a high concentration there is a transient leukocytosis followed by a rapidly developing leukopenia, thrombopenia, and anemia. The bone marrow becomes aplastic. First the platelets are reduced following repeated exposures to low concentrations.

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Clinical and Hematologic Features

The idiopathic form of aplastic anemia occurs most frequently in young adults, being rare in infants and children and infrequent in elderly persons. The secondary form may occur at any age. The onset and course are extremely variable. A rapidly progressive anemia may develop and terminate

fatally within a few weeks or days after the onset of symptoms. In other patients the onset is insidious, the course is chronic, and the manifestations of weakness and pallor may persist in a mild degree for months before they become severe. The term *hypoplastic anemia* has been applied to these chronic cases. The acute form of the disease is more common in young adults whereas the chronic variety usually occurs in older patients. When the anemia progresses very slowly the patient may be quite active in spite of an extremely low erythrocyte count.

In some patients the symptoms may be only those of anemia, such as weakness, fatigability, listlessness, shortness of breath, and palpitation. In other instances, because of the reduction in the number of blood platelets, hemorrhagic tendencies appear. These are frequently the first manifestations of the disease. The bleeding tendencies may assume many different forms, like those encountered in thrombopenic purpura. In most instances there will be hemorrhages into the skin at some stage of the illness. These may occur as repeated crops of small purpuric spots of intracutaneous hemorrhage or as larger ecchymotic areas of subcutaneous bleeding. Bleeding from the gums is very common, and there may be mild or severe hemorrhage from any part of the gastrointestinal tract. Hematuria is infrequent, but uterine hemorrhages are of frequent occurrence and may be the first indication of the illness. The anemia in these patients is not dependent on the loss of blood although hemorrhage increases the degree of anemia and accelerates the progress of the disease. Sudden loss or blurring of vision is a frequent complaint, the explanation for which is found in the retinal hemorrhages that are so prone to occur (Fig. 27). Sometimes there is cerebral hemorrhage. The hemorrhagic tendencies are less frequent and less severe in chronic cases.

These patients are extremely susceptible to infections of all types because of the leukopenia. Sepsis with chills, fever, and profuse sweating may be a predominant feature of the illness throughout its entire course. Infections may be localized in any part of the body but are particularly common about the mouth, pharynx, and upper respiratory tract. Necrotic ulcerations in the mouth and pharynx are frequent, and bronchopneumonia is often the mode of termination.

Physical examination reveals a marked pallor but no icterus and no evidence of increased hemolysis of erythrocytes. The lymph nodes are not enlarged, nor are the liver and spleen increased in size. Although mild paresthesias are occasionally noted by some patients with a chronic form of the disease, there are no evidences of sclerosis of the central nervous system. Evidences of hemorrhage into the skin or subcutaneous tissues may be pres-

ent, and there may be signs of a complicating infection in some part of the body. Although the gums may bleed profusely, there is no infiltration or piling up of the gum margins as is seen in leukemia.

Achlorhydria is not common.

Examination of the blood shows a parallel reduction in the erythrocyte count and hemoglobin level so that the color index and mean corpuscular hemoglobin remain about normal. As there is no significant change in the

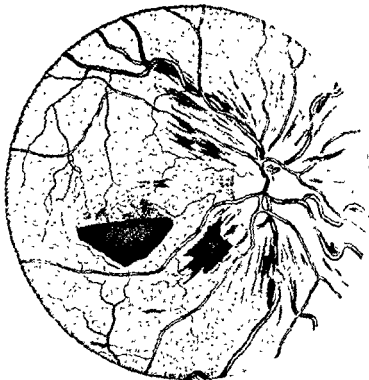


FIG. 27 Retina in a patient with aplastic anemia showing the hemorrhagic manifestations to be found in ophthalmoscopic examination

size of the erythrocytes, the volume index and mean corpuscular volume are normal. The anemia is steadily progressive without a tendency toward remissions, and an extremely low erythrocyte count is ultimately reached, frequently below 1,000,000. There are few or no evidences of regeneration of the erythrocytes. The number of reticulocytes is characteristically low, or these cells may be entirely absent; a few are encountered in some cases. Polychromatophilic erythrocytes are not present on the blood smear, and the occurrence of nucleated erythrocytes is exceedingly rare. The erythrocytes

appear essentially normal on the stained smear with but slight variation in their size and staining reactions.

The leukocyte count is low, usually ranging from 1500 to 3000 cells per cubic millimeter. Not infrequently it drops below 1000, but only on rare occasions will it be higher than 3000. The leukopenia is due primarily to a reduction in the number of neutrophils so that there is a relative lymphocytosis. The percentage of band or nonfilamented neutrophils is characteristically reduced. Immature cells of the myeloid series have been found occasionally in spite of the extremely low leukocyte count. When the total leukocyte count becomes very low, there is a reduction in the number of lymphocytes as well as granulocytes. This is obviously true when the total leukocyte count is below 1000 as such a low count could not be obtained if lymphocytes were present in normal numbers.

The platelets are always reduced in number. This accounts for the hemorrhagic features of the disease. With the thrombopenia there is a prolonged bleeding time, a nonretractile clot, and a positive constrictor or Rumpel-Leede test. These represent all the essential diagnostic features of thrombopenic purpura. They are a part of the picture of aplastic anemia and are not to be regarded as complications.

Sternal puncture in aplastic anemia is unsatisfactory in many instances since the pathology, as previously noted, is variable. If few immature nucleated cells are found it is difficult to tell whether this represents aplasia of the marrow or a liberal admixture of peripheral blood in the aspirated material. The latter is of frequent occurrence in marrow aspiration. This procedure is of greater value in excluding leukemia and other blood dyscrasias from the diagnosis than in obtaining positive evidence of aplastic anemia.

Diagnosis

The diagnosis of aplastic anemia is established by the presence of an anemia, leukopenia, and thrombopenia and by a careful exclusion of other types of severe anemia. There is no jaundice and no evidence of increased hemolysis of the erythrocytes so that hemolytic anemias can be readily excluded. The anemia is steadily progressive and more severe than can be accounted for by the amount of blood lost by hemorrhage, and the hemorrhagic anemias do not have a leukopenia or thrombopenia. Careful attention must be given to the recognition of atypical cases of pernicious anemia so that they are not mistaken for aplastic anemia and deprived of the benefits of specific treatment. In agranulocytosis the leukocytes are reduced in number, but the platelets and erythrocytes are essentially normal. In thrombopenic

purpura the leukocyte count is normal or slightly elevated, the anemia is no more severe than can be accounted for by the hemorrhage, and there are evidences of regeneration of erythrocytes. Although the spleen, liver, and lymph nodes are not enlarged in aplastic anemia, considerable difficulty may be encountered in ruling out a leukemia of the aleukemic type. This is particularly true in children, in whom the aleukemic form of lymphocytic leukemia is common. The splenic enlargement and lymphadenopathy are of help in distinguishing these cases. Final diagnosis will frequently rest on the histologic examination of bone marrow obtained by sternal puncture or biopsy. This may show a reduction in the erythrocytic and myeloid elements in cases of aplastic anemia whereas an overgrowth of immature cells of one of the leukocytic series will be apparent in leukemia.

Prognosis

The prognosis is poor. Idiopathic aplastic anemia is almost invariably fatal, death being due to the progressive anemia, to a severe hemorrhage, or to an intercurrent infection. The acute forms may be fatal within a few weeks of the onset; in the chronic type the patient may live for twelve or eighteen months while receiving repeated transfusions. Occasional cures are obtained in the secondary form of aplastic anemia if the causative agent is recognized and removed before the disease has become too far advanced. The outlook is poor if the anemia is severe or if hemorrhages have begun.

Treatment

The treatment of aplastic anemia is not satisfactory. The only procedure of significant benefit is the administration of repeated blood transfusions, given in sufficient amounts to bring the erythrocyte count to a level approaching normal and then at the necessary intervals required to maintain the patient in a comfortable state. In the acute forms of the disease the anemia progresses rapidly in spite of repeated transfusions, and death may occur within a short time. In patients in whom the disease is more chronic and more slowly progressive the blood may be built up to fairly high levels, and by means of transfusions at seven to ten day intervals the patients may be carried along for a period of months. The outcome is eventually fatal in most cases. One patient under our observation was able to continue with his work for over a year while receiving blood transfusions of 1000 cc. administered at approximately three week intervals. The use of stored blood from the blood bank seemed to be as efficacious as fresh blood in maintaining his erythrocyte level.

A few patients with aplastic anemia of the secondary type recover even though the anemia has become severe. For this reason all patients with either the idiopathic or the secondary type should be removed from contact with all possible causative agents and given repeated transfusions in the hope that if they are maintained on borrowed blood for a long enough time, their bone marrow function may return.

Neither liver extract nor iron are of benefit to these patients. Since there is always the possibility of an error in diagnosis, we give liver extract an adequate therapeutic trial in every case but discontinue its administration if there is no reticulocyte response or other evidence of improvement. All procedures and drugs which might stimulate the bone marrow have been tried without beneficial effect. These include liver extract, iron, nucleic acid derivatives, bone marrow extracts, and small doses of roentgen rays. Injections of adrenalin temporarily increase the erythrocyte count but are of no permanent benefit.

CONGENITAL HYPOPLASTIC ANEMIA

Congenital hypoplastic anemia or aregenerative anemia of infants is a disease appearing soon after birth which is characterized by an anemia with few or no evidences of erythrocytic regeneration. It resembles aplastic anemia in that reticulocytes are lacking, but leukocytes and platelets are normal or but slightly lowered. There are no hemorrhagic tendencies.

The characteristic feature is a slowly progressive anemia appearing in early infancy which does not respond to any type of therapy. The erythrocyte count may drop to extremely low levels, producing pallor, weakness, and listlessness. The liver and spleen are not enlarged and there is no lymphadenopathy. A number of these cases have been reported in which there is congenital aplasia of the marrow associated with other congenital abnormalities such as microcephaly, testicular hypoplasia, convergent strabismus, exag-

Few or no reticulocytes are present. Polychromatophilia is not evident. There is no evidence of increased blood destruction. In most cases the total leukocyte count is normal or but slightly lowered, and the differential count is not significantly altered. Only occasionally are neutropenia and thrombopenia encountered. Examination of the sternal marrow shows a reduction in the number of nucleated erythrocytes.

The anemia is chronic and progressive and can be controlled only by re-

peated transfusions. When it is kept under control by this means, the child will grow and develop normally. The disease terminates fatally if transfusions are not given, but the child can be carried along indefinitely by this means. One patient apparently recovered after two years of treatment. Transfusions should always be given in the hope that the bone marrow may ultimately regain its normal function.

The cause of this type of anemia is not known, but Diamond and Blackfan consider two possibilities (1) It may be a congenital insufficiency of the hematopoietic bone marrow rendering it unable to form a sufficient number of new cells or (2) it may represent an inborn error of metabolism of one of the blood-building substances.

EQUINE INFECTIOUS ANEMIA

A form of anemia which is similar to aplastic anemia, except for a normal platelet level, has been reported as being transmitted from the horse to man. It is due to a filterable virus and is transmitted by the bite of a fly or mosquito. It is characterized by a chronic anemia and leukopenia but a normal platelet count and by recurring bouts of fever. The diagnosis is established by injection of serum of the suspected patient into a healthy young horse.

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MYELOPHTHISIC ANEMIAS AND ANEMIAS SECONDARY TO OTHER DISEASES

MYELOPHTHISIC ANEMIAS

MYELOPHTHISIC ANEMIAS ARE THOSE CAUSED BY DESTRUCTION OF THE erythropoietic portion of the bone marrow or its replacement by other types of cells or tissue. They result from a wide variety of lesions which produce different types of reactions in the marrow. These may be purely destructive in character, or there may be compensatory hyperplasia in the surrounding marrow tissue resulting in the appearance of immature cells in the blood stream. The effect of the lesions on the leukocytes and platelets is variable so that there is no constant hematologic picture in myelophthisic anemia. Other factors besides the destruction of bone marrow may contribute to the anemia so that it may not be purely myelophthisic in origin.

Causes

Metastatic Carcinoma

A malignant tumor in itself does not cause anemia except by its secondary effect on the nutritional state, by loss of blood, and perhaps by a toxic depression of the bone marrow. When malignant growths metastasize to the bone marrow, the anemia becomes more severe and more rapidly progressive. Metastases to bone are most apt to occur with a carcinoma of the prostate, breast, lungs, or thyroid but may occasionally occur when the primary lesion is in another organ. Carcinoma of the prostate has been the most frequent offender in our experience. In many instances the anemia with its associated weakness, pallor, and breathlessness has preceded any symptoms referable to the primary lesion. The pelvic bones are the most common site for metastases from the prostate (Fig. 28)

With extensive metastases to the bone marrow there is a progressive anemia of variable severity which is commonly hypochromic in type although it

may be normochromic or even hyperchromic. This may become extremely severe. The leukocyte count is usually normal or but slightly decreased although occasionally a definite leukopenia will be found. Nucleated erythrocytes are frequently encountered. These are a result of irritation rather than evidence of blood regeneration. There may be immature cells of the granulocytic series in the blood stream. In some cases these are numerous enough



FIG 28. Extensive osteolytic lesions in the pelvis and femur as a result of metastases from a carcinoma of the prostate

to raise the question of leukemia in the differential diagnosis. The platelets are not significantly affected as a rule but may be decreased in number. A picture of aplastic anemia is presented when all the marrow elements are decreased although demonstration of the primary lesion and roentgenologic evidence of the metastatic lesions suffice for differentiation. A leukemoid reaction with leukocytosis, immature myeloid cells, and nucleated erythrocytes may occur.

Neither iron nor liver are of value in the treatment of this anemia. Transfusions give only temporary relief.

Leukemia

A variable degree of anemia is a constant feature in leukemia. The primary cause of this myelophthistic anemia is usually obvious from examination of the blood smear, but histologic study of the bone marrow or a lymph node may be necessary. The diagnosis may not be obvious in the aleukemic forms of leukemia with a normal or low total leukocyte count and relatively few abnormal cells in the blood stream. In many such cases the outstanding clinical feature is a steadily progressive anemia which has not responded to the ordinary methods of treatment.

The anemia is usually severe in patients with acute leukemia and progresses rapidly until the patient is troubled quite as much by the symptoms of anemia as by the other manifestations of the disease. The leukocyte count may be low, normal, or high, and varying numbers of immature cells will be found in the blood stream. The number of platelets will be reduced in most cases, and the other clinical and laboratory manifestations of thrombopenic purpura will appear. Hemorrhage may aggravate the already severe anemia. The reduction of the hemoglobin, the hematocrit, and the erythrocyte count are of about the same degree so that the color and volume indices remain normal. An increased fragility of the erythrocytes has occasionally been noticed. This may be accompanied by an increased serum bilirubin indicating an excessive hemolysis of erythrocytes so that replacement of the bone marrow is probably not the only factor in the production of the anemia associated with leukemia. We have observed one patient in whom there was an extensive leukemic infiltration of the marrow before any reduction in the erythrocyte count or hemoglobin level was apparent in the peripheral blood and the patient's only symptom was an unexplained fever. The severe anemia which ultimately develops is frequently the cause of death.

In the chronic forms of leukemia the anemia is less severe and less rapidly progressive. In the early stages, even though the leukocyte count is very high, the hemoglobin and erythrocyte levels may be well maintained, and only a moderate degree of anemia may be present. The volume and color indices usually remain normal or are only slightly reduced unless the picture is complicated by hemorrhage. In the late stages the anemia becomes more severe. Nucleated erythrocytes may be present in great numbers from time to time during the course of either the acute or the chronic form of the

disease. These "showers" of nucleated erythrocytes are not indicative of regeneration or improvement of the anemic state.

Hodgkin's Disease

Hodgkin's disease, the sclerosing type of malignant lymphoma, is always accompanied by anemia, slight or absent in the early stages but appearing as a slowly progressive anemia in the late stages. There is little or no change in the size or hemoglobin content of the erythrocytes. Infiltration of the bone



FIG. 29 Roentgenogram of the skull showing extensive mottling as a result of hyperparathyroidism.

marrow by lymphomatous tissue is of common occurrence; when this becomes extensive, the anemia progresses more rapidly. In a few cases of Hodgkin's disease the pathologic changes are confined mainly to the spleen or to the bone marrow with little or no lymph node involvement. A severe anemia is the principal clinical feature in such patients.

Hyperparathyroidism

Hyperparathyroidism, due to adenomas or hyperplasia of the parathyroid glands, results in decalcification of the skeletal system and the formation of multiple bone cysts—osteitis fibrosa cystica (Figs. 29 and 30). A severe grade

of anemia occasionally develops as a result of this widespread involvement of bone with replacement of the normal marrow by fibrosis and multiple cysts. It is sometimes accompanied by a leukopenia and thrombopenia so that a blood picture resembling aplastic anemia is produced. The clinical features and the chemical studies of the blood serum differentiate it from other forms



FIG. 30 Roentgenogram of a femur in a case of hyperparathyroidism showing osteitis fibrosa cystica.

of myelophthitic anemia. There is great muscular weakness, hypotonia, anorexia, polydipsia, polyuria, and pains in the skeletal system. Renal colic due to nephrolithiasis is common. The blood shows an elevated serum calcium, a decrease of the serum phosphorus, and an increase in phosphatase activity.

Multiple Myeloma

Multiple myelomas are tumors arising in the bone marrow. The plasma cell type (plasmocytoma) is the most common but myeloblastomas and

erythroblastomas may also occur. Roentgenograms show widespread osteolytic lesions which are found most often in the pelvis, skull, and ribs.

The hematologic picture is identical in all types of myeloma. Immature leukocytes do not ordinarily appear in the blood stream. In a few instances a leukemoid reaction may occur with plasma cells or other leukocytes predominating. The anemia is at first mild but progresses steadily. The reductions in the hemoglobin and erythrocyte levels are about equal so that normal color and volume indices are maintained. Excessive rouleau formation of the erythrocytes is occasionally encountered, and the sedimentation rate is very rapid. Sternal aspiration will frequently show myeloma cells in the aspirated material even though there is little X-ray evidence of the disease and the procedure should be carried out in all unexplained progressive anemias.

Lipoid Dystrophies

The lipid dystrophies or xanthomatoses comprise a group of diseases in which there is an abnormal metabolism of lipid substances resulting in a deposition of these substances in the reticulo-endothelial cells throughout the body. A myelophthisic anemia develops when the lipid-containing reticulo-endothelial cells replace the active hematopoietic cells of the bone marrow.

Gaucher's Disease. Gaucher's disease is a chronic form of lipid dystrophy in which kersin, an abnormal lipid substance, is found in the reticulo-endothelial cells. The spleen becomes enormously enlarged, and there is a generalized rarefaction of the bones with thinning of their cortex. A mild anemia and leukopenia are characteristic.

Niemann-Pick Disease Niemann-Pick disease begins earlier in life and pursues a more rapid course than Gaucher's disease. The abnormal metabolite is a phosphatide rather than kersin. A leukocytosis has been encountered more frequently than a leukopenia in this disease.

Hand-Schuller-Christian Disease In Hand-Schuller-Christian disease the reticulo-endothelial cells contain cholesterol, and the blood cholesterol level may be elevated. Exophthalmus, diabetes insipidus, and rarefied areas in the membranous bones are the characteristic findings. The bone lesions, which are most numerous in the skull, are found to consist of degenerated cells containing the lipid material. The hematologic features are frequently suggestive of aplastic anemia.

Albers-Schonberg Disease

Synonyms: Osteopetrosis, osteosclerosis fragilis, marble bones

Albers-Schönberg Disease is a rare familial disease characterized by in-

creased thickness and density of the cortex of the bones with encroachment on, and at times almost complete obliteration of, the marrow cavity (Fig. 31). The bones are unusually fragile so that fractures are common. It occurs most frequently in children but is also found in adults.

The skeletal changes are accompanied by a variable degree of anemia which does not necessarily parallel the degree of bone involvement as demonstrated roentgenologically. Polychromatophilic cells and nucleated erythrocytes are occasionally found in the blood stream. A leukocytosis with immature myeloid cells is apt to occur in infants with this disease, but the



FIG. 31 Roentgenogram of the arm of a 2-year-old child showing osteopetrosis or marble bones. Necropsy revealed a thickened cortex with sclerosis and partial obliteration of the marrow cavity. Hemoglobin 5.1 Gm per 100 cc., erythrocytes 2,140,000, and leukocytes 11,500.

changes in the blood are usually less striking in adults. The spleen and liver are commonly enlarged.

Although anemia is the most constant hematologic feature of this disease, there are many reports in the literature of a leukemic blood picture occurring in association with a diffuse sclerosis of the skeletal system.

ANEMIAS SECONDARY TO OTHER DISEASES

Causes

Carcinoma

The anemia which so frequently accompanies carcinoma varies a great deal in its severity, depending upon the type and location of the primary lesion and upon contributory causes for anemia which may appear. The mere pres-

ence of a neoplasm does not cause anemia but owing to hemorrhage, ulceration, secondary infection, or the malnutrition which it produces, an anemia of variable severity commonly develops. When the neoplasm involves the bone marrow, a myelophthitic anemia appears; when it involves the esophagus, stomach, pancreas, or other portions of the gastrointestinal tract, the effect on the nutritional state of the individual is such that anemia supervenes. Hemorrhage is a contributing factor in the production of the anemia in a great many cases.

Carcinoma of the stomach is prone to cause a particularly severe and rapidly progressive anemia. There is, in a majority of the advanced cases, a constant loss of small or large amounts of blood. Coupled with this loss of blood is the malnutrition resulting from anorexia and indigestion. In addition to these features there is, in a few cases, a disturbance of the secretory function of the gastric mucosa. In the absence of the intrinsic factor from the gastric secretion the antianemic or maturation factor for regulating erythropoiesis is not formed. This leads to a macrocytic type of anemia resembling pernicious anemia whereas the anemia resulting from chronic blood loss is microcytic in type.

The administration of iron will aid in the control of the anemia which is due to loss of blood but cannot be expected to maintain a normal hemoglobin level. The administration of liver extract should aid in combating the anemia which is due to a deficiency of the intrinsic factor, but we have encountered none in whom this type of therapy produced a significant response. Transfusions give temporary benefit by increasing the sense of well-being of the patient but do not alter the course of the disease.

Carcinoma of the colon is also prone to produce a particularly severe grade of anemia because of the constant loss of blood. Local symptoms and changes in bowel habits are apt to be absent or very slight when the growth is located in the ascending portion of the colon. The bleeding is high enough in the intestinal tract so that the blood becomes intimately mixed with the feces and is not noticed by the patient. A severe grade of anemia develops in many instances before there are any localizing symptoms of the carcinoma. A careful analysis of the feces for blood must be a part of the examination in all cases of hypochromic and microcytic anemia. Although the administration of iron may aid in the control of this anemia, it cannot compensate for the continued loss of blood.

Carcinoma of the liver, either in a primary or in a secondary metastatic form, may interfere with the hematopoietic function of that organ to such an extent that anemia becomes a prominent feature.

Hypothyroidism

A high percentage of patients having a deficient thyroid secretion or myxedema will develop an anemia. In many the anemia is mild and plays no part in the patient's symptoms. In other instances a severe anemia develops which may be either hypochromic or hyperchromic in type. This anemia may be associated with a leukopenia, but usually the leukocytes and platelets are normal. Free hydrochloric acid is absent from the gastric contents of 50 per cent of myxedematous patients, according to Lerman and Means, but in our experience the percentage has been even higher. A blood picture which is suggestive of pernicious anemia has occasionally been observed, and in a few instances true pernicious anemia has been found associated with myxedema. When this latter combination is present, the pernicious anemia will respond to the administration of liver extract even though thyroid extract is withheld, indicating that the bone marrow is capable of a normal reaction. The anemia associated with hypothyroidism is due to a depression of bone marrow function, a part of the general lowering of the body metabolism. This condition should be considered in the differential diagnosis of all mild hypochromic anemias which are not otherwise explained.

The administration of thyroid extract controls the manifestations of hypothyroidism, and the anemia gradually improves. The improvement appears to be slightly more rapid if an inorganic iron salt is administered. Liver extract, in the absence of pernicious anemia, is of no value.

Chronic Nephritis

An anemia is commonly associated with chronic glomerulonephritis even though there is no nitrogen retention. This is usually mild with the hemoglobin and erythrocyte count being proportionately reduced so that the color index is about normal. The anemia apparently does not depend upon loss of blood into the urine or to an excessive hemolysis of erythrocytes. It is probably due to a toxic depression of the bone marrow as there are but slight evidences of blood regeneration. It is usually mild in the early stage of the nephritis but increases as the renal damage progresses. A very severe anemia may ultimately develop.

Chronic Nitrogen Retention

A moderate to severe grade of anemia is found in association with the retention of nitrogenous waste products in the blood. The degree of anemia roughly parallels the severity of the nitrogen retention. The blood hemoglobin and the erythrocyte count are proportionately reduced in the early

ence of a neoplasm does not cause anemia but owing to hemorrhage, ulceration, secondary infection, or the malnutrition which it produces, an anemia of variable severity commonly develops. When the neoplasm involves the bone marrow, a myelophthisic anemia appears; when it involves the esophagus, stomach, pancreas, or other portions of the gastrointestinal tract, the effect on the nutritional state of the individual is such that anemia supervenes. Hemorrhage is a contributing factor in the production of the anemia in a great many cases.

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Carcinoma of the liver, either in a primary or in a secondary metastatic form, may interfere with the hematopoietic function of that organ to such an extent that anemia becomes a prominent feature.

Chronic Infections. Chronic infections are associated with varying degrees of anemia which, as a rule, are mildly hypochromic in type. The severity of the anemia depends to some extent upon the type of invading organism and upon the location of the infection. Septicemia, especially with a hemolytic streptococcus, will cause a particularly severe grade because of the excessive hemolysis of erythrocytes. Certain other infections, such as acute rheumatic fever, subacute bacterial endocarditis, *B. welchii* infections, chronic tuberculosis, and osteomyelitis, are particularly prone to cause an anemia. In other chronic infections the degree of anemia is usually moderate, but this depends to a great extent on the severity of the infection, its duration, and on whether or not there are other contributory factors that may accentuate the anemia.

The cause of the anemia is not well understood. From the evidence at hand it seems probable that it is due to a depression of bone marrow function, possibly from the effects of bacterial toxins. It has been noted that an infection interferes with the therapeutic effectiveness of liver extract in the treatment of pernicious anemia, delaying or lowering the reticulocyte response. This suggests an inhibitory or depressant effect on the bone marrow. Additional factors may accentuate the anemia of infections. In some instances, as with ulcerative colitis, there is a loss of significant amounts of blood. Other instances show anorexia and digestive disturbances which interfere with the intake or absorption of adequate food. Wintrobe has shown that the anemia associated with infection is accompanied by a low plasma iron level which drops soon after the onset of infection and cannot be raised by the administration of iron orally or parenterally until the infection has subsided. The iron appears to be removed rapidly from the blood plasma by diversion to the liver and other tissues, and the anemia is caused by impaired hemoglobin formation.

The diagnosis of this type of anemia depends upon the recognition of the infectious process. It must be remembered, however, that patients are rendered more susceptible to infection by the presence of an anemia so that infection is frequently superimposed upon a preexisting anemia. A febrile reaction not uncommonly accompanies a severe grade of anemia and subsides to normal upon adequate therapy. Thus the presence of a mild febrile reaction does not necessarily indicate a complicating infectious process.

Treatment consists in eradication of the infection. If this can be accomplished, there is no need for further therapy although recovery may be hastened to some extent by the administration of iron. Liver extract is not effective in treating this type of anemia.

stages, but a low color index is usually found in the more severe cases. In a few instances the anemia is hyperchromic in type. There may be some variation in the size of the erythrocytes, but variations in their shape are not striking. There is no elevation of the reticulocyte count, the resistance of the erythrocytes to hypotonic saline solution is normal, and there is little or no evidence of excessive hemolysis of erythrocytes. The anemia is apparently due to a diminished production of hemoglobin and erythrocytes as a result of a toxic depression of the bone marrow. It is not particularly suggestive of aplastic anemia, however, since there is usually no reduction in the number of leukocytes or platelets. This type of anemia will be associated with the nitrogen retention of chronic nephritis as well as with that accompanying prostatic obstruction, polycystic kidney, or any disease leading to increased blood nitrogen. Hemorrhagic tendencies are prone to develop and profuse bleeding from any part of the body may occur. Although the bleeding may be profuse there is no alteration in the bleeding time, coagulation time, prothrombin time, clot retraction, or platelet count.

The treatment of this anemia is unsatisfactory. Its alleviation depends upon relieving the nitrogen retention, and this cannot be accomplished in many patients unless it is due to a blockage of the urinary tract which can be removed. The administration of neither liver nor iron is of value. Transfusions may be given for palliation, but the danger of transfusion reactions is greater in patients with reduced renal function than in those with normal kidneys.

Infection

Acute Infections Acute infections are commonly associated with varying degrees of anemia, the severity depending upon the type of invading organism and upon the extent and distribution of the infection. Even the common acute upper respiratory infections usually cause a transient anemia in which there is a rather sudden fall in the blood hemoglobin and erythrocyte count with a very gradual return to normal value. Such a reaction was frequently encountered while following the rate of hemoglobin regeneration in blood donors. It was noted that the occurrence of an upper respiratory infection slowed the rate of hemoglobin regeneration or produced a drop in the hemoglobin level which might equal that occurring with the blood donation. Although the mode of production of such an anemia is variable, the suddenness of the hemoglobin drop suggests that there is hemolysis of erythrocytes. It is apparently not entirely due to inhibition of the bone marrow resulting from the infection although this may play a part.

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Vitamin C Deficiency

A deficiency of vitamin C, both in adults and in children, leads to the development of scurvy. This, in the fully developed case, is characterized by pallor, spongy and bleeding gums, and by petechiae, ecchymoses, and hemorrhages elsewhere in the body. The anemia which is a part of the clinical picture is probably due largely to the loss of blood. It is of the hypochromic type.

There are a few cases of vitamin C deficiency in which the typical picture of scurvy is not present. In spite of the fact that there is no evidence of blood loss in these cases, there is an anemia of varying severity. The exact role of vitamin C in the production of erythrocytes is unknown and there is some question as to whether or not its absence actually causes an anemia without the appearance of clinical scurvy. Some observers have reported a prompt recovery following the administration of cevitic (ascorbic) acid but others deny the existence of such an anemia.

Hookworm Infestation

Infestation by hookworms is prevalent in tropical and subtropical countries. Many of the symptoms which characterize the clinical course of this disease are due to the anemia which is always present in patients harboring a large number of the organisms. Weakness, loss of pep, and fatigability together with gastrointestinal symptoms such as anorexia, nausea, vomiting, constipation, and diarrhea are the outstanding symptoms. The diagnosis is based on the presence of ova or worms in the intestinal contents.

The anemia is brought about primarily by the continuous loss of small amounts of blood in the feces which ultimately results in the development of an iron deficiency anemia. In some instances a severe irreversible aplastic type of anemia has developed but it is difficult to separate the blood loss, the dietary deficiency and other factors as causative agents.

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circulatory collapse are similar to those of ordinary shock which accompanies an injury except that restlessness, air hunger, and thirst occur with the shock resulting from the loss of blood.

The fall in blood pressure is due to the decrease in cardiac output which results from the diminished blood volume and venous return. If the patient survives the first critical stage, the blood pressure is gradually raised through the efforts of the vasomotor center resulting in vasoconstriction. This, together with an acceleration of the heart rate, increases the blood supply to the vital centers. The increased heart rate is initiated by a reflex action from the fall in blood pressure. A rate of from 120 to 140 beats per minute is common. The high rate persists even after the blood pressure has been raised, remaining fast throughout the time that a severe anemia persists. During this stage the tachycardia is due to anoxemia resulting from the lowered oxygen-carrying power of the blood.

The fluid balance between tissues and vascular system is disturbed by the lowered blood volume and incomplete filling of the vascular bed. Fluid passes from the tissues to the blood stream, and the normal blood volume is restored. This results in tissue dehydration and causes the sunken facies and increased thirst. The blood volume is restored to normal by dilution with a fluid which is low in its protein content. The plasma proteins are thereby lowered and may become so diluted that their effect on the osmotic pressure of the blood is reduced. This causes fluid from the blood stream to revert to the tissues, and edema may result. Whereas the first fluid which replenishes the blood volume is low in its protein content, that which comes in after the first two hours contains an increased amount. The plasma proteins are thereby replenished in the later stages of the recovery period and gradually return to their normal level.

The hematocrit reading gradually falls after a hemorrhage while the blood volume is being restored and the blood is being diluted with fluid. The hematocrit will have reached its lowest level at the end of twenty-four hours unless the hemorrhage has been extremely severe.

The hematologic findings will depend upon the length of time intervening between the hemorrhage and the examination. There will be little or no deviation from the normal if an erythrocyte count and hemoglobin determination are made soon after a hemorrhage. The only change encountered at this time is in the blood volume with no significant disturbance in the concentration of the hemoglobin or erythrocytes per unit volume of blood. There is a gradual fall in the hemoglobin concentration and the erythrocyte count as the blood volume becomes readjusted during the following hours. This

ANEMIA DUE TO ACUTE BLOOD LOSS. BLOOD DONORS

ANEMIA OF ACUTE BLOOD LOSS

THE IMMEDIATE EFFECTS OF A PROFUSE HEMORRHAGE DEPEND NOT ONLY UPON the amount of blood lost but also upon the rapidity with which it escapes. The symptoms are due primarily to the reduction in blood volume rather than to the reduced number of erythrocytes. When the loss of blood takes place very slowly or when there is an intermittent loss of small amounts of blood, the patient may ultimately develop a very low hemoglobin level and erythrocyte count and still have comparatively few symptoms. When the loss of blood occurs within a short period of time, syncope, collapse, or death may result from the loss of one-third to one-half of the blood volume. The removal of from 15 to 19 per cent of the blood volume (760 to 1220 cc.) caused circulatory collapse in 5 of 6 subjects, and 1 became unconscious, according to Ebert, Stead, and Gibson.

The symptoms and manifestations of acute hemorrhage differ greatly from one patient to another even when the blood loss is approximately equal. The removal of fairly large amounts of blood for experimental purposes resulted in few or no symptoms until the onset of circulatory collapse. This occurred suddenly, either during or soon after the removal of the blood. The immediate symptoms of any severe acute hemorrhage consist of weakness, nausea, blurred vision, pallor, and profuse sweating. There is first an acceleration of the heart rate, but with the onset of circulatory collapse the heart becomes slow with a rate of 36 to 40 beats per minute. A precipitous drop occurs in both the systolic and the diastolic blood pressures. The pulse becomes weak and thready. There is an ashen pallor of the skin, which becomes cold and covered with beads of perspiration. This acute collapse may result in unconsciousness or may be fatal. Varying degrees of prostration follow, and there is increased thirst, restlessness, and air hunger. The symptoms of this

having some chronic disease such as nephritis, hypothyroidism, Banti's syndrome, or infection. An acute infection during the recovery period will also delay the regeneration of hemoglobin and erythrocytes

Treatment

The most important feature in the management of a patient after severe hemorrhage is the treatment of shock and the restoration of a normal blood volume. This can best be accomplished by transfusions of whole blood since they not only increase the blood volume but also aid in restoring the erythrocytes and hemoglobin to their normal levels. If whole blood is not available, the administration of blood plasma or the use of dried serum is advisable since these restore the erythrocyte and serum protein levels. Saline or glucose solutions may be given intravenously if blood, plasma, or serum are not available, but they are less effective, and the increased blood volume resulting from their use is transient.

Spontaneous recovery with restoration of normal hemoglobin and erythrocyte levels will take place so that specific treatment of the anemia is not imperative. The administration of iron salts will, however, hasten the regeneration of hemoglobin. It is not necessary to employ the large doses which are recommended for iron deficiency anemias since smaller ones such as 1 to 1.5 Gm. per day of either iron and ammonium citrates or reduced iron or 1 Gm. of ferrous sulfate will produce an adequate response.

ANEMIA DUE TO CHRONIC BLOOD LOSS

Although hemoglobin will be regenerated rapidly and spontaneously after a single large hemorrhage, this does not hold true when blood is lost repeatedly over a long period of time. There is an adequate amount of iron in the body for hemoglobin regeneration after a single hemorrhage, but an iron deficiency develops and hemoglobin production becomes slower when the available iron is used more rapidly than it is being replaced. This type of hypochromic microcytic anemia is discussed with the iron deficiency anemias. How much blood can be lost and over what period of time without a deficiency of iron developing cannot be ascertained. It unquestionably varies with the individual. Healthy blood donors are capable of giving repeated donations over a long period of time without evidence of a lessening of hemoglobin regeneration provided the hemoglobin is allowed to return to normal between donations.

reaches its lowest level after twenty-four to seventy-two hours. Blood counts and hemoglobin determinations are of no value in estimating the amount of blood lost until after the blood volume has been completely restored. The continual downward trend is not an indication of continued bleeding. When only a small amount of blood is lost, as in the case of a blood donation for a transfusion, the fluid loss is made up within a few hours, and even after a fairly large hemorrhage the blood volume is usually back to normal within twenty-four hours. The rapidity with which fluid is replaced in the blood stream varies with the availability of tissue fluids so that in fat, plethoric individuals the fluid is replaced more rapidly than in those who are thin. The replacement may be hastened by giving large amounts of fluid by mouth or by parenterally administered fluids.

There is an increase in the number of blood platelets, which begins soon after the hemorrhage and reaches a peak several days later. The number subsequently recedes to the normal level. There is also an increase in the leukocyte count, which reaches its peak on the fourth or fifth day after a hemorrhage. The height which the leukocyte count may attain is variable but may reach 30,000 or more. The elevation of the total white count is due to the increased number of neutrophils and is accompanied by a nuclear shift to the left.

After twenty-four to forty-eight hours there are indications that regeneration of erythrocytes is beginning. This is first manifested by an increase in the number of reticulocytes, which gradually reaches a peak on the tenth to twelfth day and then recedes to normal level. The height of the peak is roughly proportional to the amount of blood lost but seldom goes above 12 or 15 per cent. The leukocytes, platelets, and reticulocytes are usually back to their normal levels after fourteen days. An occasional normoblast and a few polychromatophilic erythrocytes may be found in the blood stream during the period of erythrocyte regeneration, but even after these evidences of bone marrow activity have disappeared, there is a progressive increase in the hemoglobin and number of erythrocytes. This increase is not a steady progression, it is more rapid in the early stages of the recovery period and becomes slower as the normal level is approached. Erythrocytes are regenerated more rapidly than is the hemoglobin so that the cells become somewhat hypochromic and the color index falls below normal. There are great individual variations in the rapidity of blood regeneration, the length of the recovery period varies from one person to another even with the loss of equal amounts of blood. The rate of regeneration is slower in patients

and also that the average recovery period is longer than is generally supposed for such a relatively minor blood loss. The accompanying chart (Fig. 32) shows the percentage of cases whose hemoglobin returned to its original level during each seven day period following a blood donation. It will be noted that at the end of eight weeks the hemoglobin of only 75 per cent of the subjects was back to its normal level. The remaining 25 per cent had not yet recovered although it has been a common practice to require an interval of only six to eight weeks between blood donations. These data show that the interval is too short to be safe unless the donors' hemoglobin is determined before each donation to be sure that recovery from the preceding donation is complete. When smaller amounts of blood are withdrawn, the drop in the hemoglobin level is correspondingly less and the recovery period is shorter.

When repeated blood donations are given, after allowing the hemoglobin to return to its original level, there is no appreciable diminution in the rate of hemoglobin regeneration. The donor is not harmed even when large numbers of donations are made provided complete recovery is allowed to take place between bleedings.

The daily increase in the blood hemoglobin under the stimulus of this mild grade of anemia was 0.0518 Gm. per 100 cc. of blood. This was slightly lower in females than in males, but the height of the original hemoglobin level did not influence the rate of regeneration.

Effect of Iron on Hemoglobin Regeneration in Blood Donors

Although there is no reason to believe that there is a deficiency in available iron in the healthy adults who serve as blood donors, it was found that the administration of an inorganic iron salt hastened the regeneration of hemoglobin to a remarkable degree for a limited period of time. The rate of hemoglobin regeneration in a group of donors was followed after one blood donation without medication. After the hemoglobin had returned to its original level, another 500 cc. of blood was taken. The rate of regeneration was again followed while the subjects received 1 Gm. of iron and ammonium citrates per day. Following the first blood donation the rate of hemoglobin regeneration was found to be 0.0518 Gm. per 100 cc. per day whereas after the second donation, while iron was being administered, the rate of regeneration was 0.772 Gm. per day. This represents an increase of 49 per cent in the rate of hemoglobin regeneration under the influence of iron therapy. The length of time required for the hemoglobin to return to its original level was correspondingly shortened. Without medication the average recov-

BLOOD DONORS

The great demand for whole blood, plasma, and serum for transfusion purposes in the past few years has resulted in an intensive study of the effects of blood loss and of the rate of hemoglobin regeneration in blood donors. Much information has been gathered on the fall in the blood hemoglobin and the rapidity of hemoglobin regeneration following the loss of a known amount of blood. In most cases of acute blood loss it is impossible to determine the exact amount which has escaped, and without the original hemoglobin level the time required for the blood hemoglobin to return to normal cannot be found. These factors can be ascertained in the blood donors. They

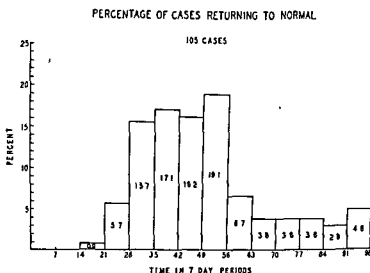


FIG. 32. The percentage of donors whose hemoglobin returned to normal in each seven day period is shown by the figures in each column. The predonation hemoglobin level was reached by only 74.7 per cent at the end of eight weeks.

have furnished the most reliable information as to the rate of hemoglobin regeneration in normal subjects.

It has been shown in a large group of donors that the removal of approximately 500 cc. of blood results in a fall in the hemoglobin level which averages 2.3 Gm. per 100 cc. of blood. The time required for the blood hemoglobin to return to its original level under these circumstances ranged from eighteen to ninety-eight days with an average recovery period of fifty days. This shows that there is a marked variation in the rate at which hemoglobin regeneration occurs in normal individuals under almost identical conditions.

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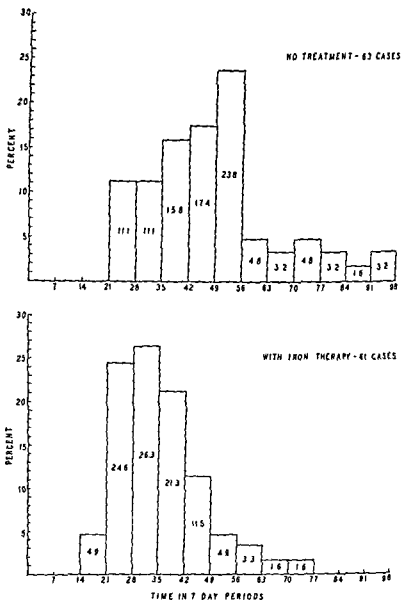


FIG. 33. This demonstrates the shortening of the recovery period in blood donors as a result of the administration of an inorganic iron salt. The figures in the columns represent the percentage of donors whose hemoglobin returned to the predonation level during the seven day period (Fowler and Barer, *J. A. M. A.*)

ery period was forty-eight days, whereas with the administration of iron it was thirty-five days, a decrease of 27 per cent. Similar effects with iron therapy are found when the drug is administered after the first donation and withheld after the second so that the results are obviously due to the iron medication and cannot be explained on the basis of erythropoietic stimulation resulting from blood loss. In the accompanying chart (Fig. 33) it will be noted that the percentage of patients whose hemoglobin returned to its original level without iron therapy gradually increased to reach a peak on the eighth week and that 78.2 per cent of this group had completely recovered by that time. With the administration of an inorganic iron salt the corresponding peak occurred in the fifth rather than the eighth week.

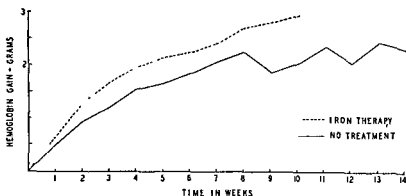


FIG 34 Showing the rate of hemoglobin increase in blood donors with and without iron therapy. (Fowler and Barer, *J. A. M. A.*)

Seventy-seven per cent of the group had recovered by the end of the sixth week and 93.5 per cent by the end of the eighth week. It is seen that the administration of iron shortens the recovery period so that the subsequent donations of blood may be given after a considerably shorter interval (Fig. 34). Various types of inorganic iron salts have been tried with equal success. Ferrous sulfate is effective in somewhat smaller amounts than iron and ammonium citrates, but when larger amounts of any of the preparations were given, they did not cause a significantly greater response.

The effect of iron on hemoglobin regeneration following acute blood loss is relatively transient. The drug becomes less effective when administered continuously throughout repeated recovery periods so that when a blood donor gives a series of donations at frequent intervals, there is progressively less effect from iron medication. During the first period of its administration the rate of hemoglobin regeneration is increased 49 per cent. During the

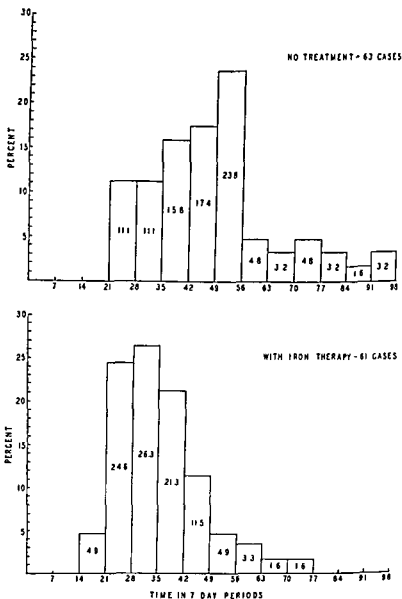


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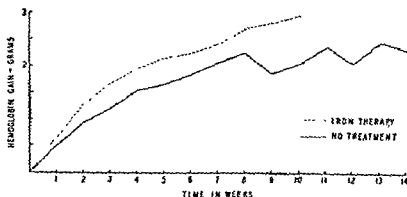


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second period the regeneration is more rapid than without medication but less rapid than when iron was first administered. After subsequent blood donations the iron has no appreciable effect on the rapidity of hemoglobin formation. When iron is administered intermittently to donors giving repeated blood donations, so that rest periods are interspersed with periods of iron medication, there is some acceleration in the rate of hemoglobin regeneration with each period of medication. The effect is not as great as that which was obtained during the first period of its administration. When donors are used repeatedly it is therefore best to administer iron during the recovery period but to stop when the hemoglobin has returned to its normal level and start again at the time of the next blood donation. In this way the maximum effect of iron can be obtained.

Vasomotor Reactions in Blood Donors

Untoward symptoms occur in a very small percentage of those individuals from whom 500 cc. of blood is withdrawn for purposes of blood transfusion. When such symptoms do appear, they are predominantly vasomotor phenomena which commonly result in weakness, faintness, and occasionally loss of consciousness. Such manifestations occur in about 5 per cent of blood donors. It is impossible to predict beforehand which subjects will faint, but it is apt to be those who have a history of previous fainting from any cause, those who are nervous before the procedure, and young individuals. Fainting is more frequent in women than in men although even a burly, husky man occasionally has such a reaction. Hunger and fatigue seem to predispose to fainting. The reaction may occur during the withdrawal of blood but is more frequent after the full amount has been taken. In a small percentage of donors the reaction is delayed until some minutes or hours have passed after the withdrawal of blood. The blood donor usually rests on a bed or couch for at least half an hour after withdrawal of blood, at the end of which time he may faint when first assuming erect posture. At the expiration of the rest period it is customary to give the donor a drink of warm tea or coffee, and occasionally the fainting occurs then. It is impossible to determine the frequency of delayed reactions since they occur when the donor is no longer under observation. The fainting may take place after leaving the blood collection center and occasionally after returning to work.

The manifestations of the vasomotor reactions vary. Many people have minor manifestations without fainting or loss of consciousness. Pallor is an almost constant feature in those who have any type of reaction and is one of the earliest manifestations. Dizziness and a feeling of faintness may occa-

sionally precede the pallor and in some cases may occur only after there is actually circulatory collapse. A transient flushing of the face and a feeling of warmth may be the first symptom in some instances. Sweating is common and may be slight or so extremely profuse as to soak the clothing. Vomiting occasionally occurs. Convulsions have occurred in a very few instances, always appearing after the premonitory signs of faintness, pallor, and loss of consciousness. The convulsions are characterized by a tonic spasm with dilated pupils followed by clonic movements of varying intensity and duration. It is possible that these represent epileptic seizures in patients with heretofore unrecognized idiopathic epilepsy, the reaction from the loss of blood being sufficient to initiate an attack. Tetany has developed in a few instances and has persisted for as long as thirty minutes.

A fall in blood pressure accompanies the fainting attack, but there is no constant relationship between the blood pressure level and the severity of the vasomotor reaction. The drop in blood pressure is considerably greater in those who faint than in the average donor. The latter shows little or no change in blood pressure as a result of removing 500 cc. of blood. A rapid pulse is frequent as a result of nervousness but a lowered pulse rate occurs with circulatory collapse.

The fainting varies in severity. It may be mild with a transitory pallor, blurring of vision, weakness, faintness, and a slight drop in the blood pressure and pulse rate. In the more severe forms there will be more pronounced pallor, profuse perspiration, and a pronounced and prolonged fall in the blood pressure and pulse rate. Vomiting, convulsions, and tetany may occur in the severe forms.

The time required for recovery from these episodes varies from a few minutes to more than an hour. It is usual for the blood pressure to return to normal first with the pallor subsequently disappearing. The opposite sequence of events is occasionally noted. Fainting may recur after the subject has apparently recovered and attempted to sit or stand.

These vasomotor phenomena occur in only a very small number of blood donors. A vast majority represent only transient and unpleasant symptoms rather than serious reactions. They present many features similar to those known to occur with "irritable heart" or neurocirculatory asthenia, and undoubtedly persons of this type are prone to have the vasomotor reactions

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HEMOLYTIC ANEMIAS

THOSE ANEMIAS WHICH ARE DUE TO AN EXCESSIVE DESTRUCTION OF ERYTHROCYTES, the hemolytic anemias, present some of the most interesting and puzzling problems in the field of hematology. The pathogenesis of many of these conditions has not as yet been firmly established. There are many parasites, infections, toxins, drugs, and chemicals which will cause hemolysis of erythrocytes, and these extrinsic factors must be excluded as etiologic agents before a diagnosis of primary or intrinsic hemolytic anemia can be made. Even after the extrinsic causes have been excluded, the mere presence of an anemia plus acholuric jaundice does not mean that there is an excessive destruction of erythrocytes since certain diseases of the liver may lead not only to jaundice of the acholuric type (without bile in the urine) but also to varying degrees of anemia. Most hemolytic agents, of either the intrinsic or the extrinsic type, do not destroy the erythrocyte directly but alter its composition or its membrane in such a way that the cell is more readily destroyed by the reticulo-endothelial tissues.

HEMOLYTIC ANEMIAS RESULTING FROM EXTRINSIC CAUSES

Destruction of erythrocytes within the blood stream may be caused by a number of extrinsic factors such as infections, infestations, toxins, and chemical agents. This destruction of cells leads to an anemia of varying degree, to jaundice when there has been sufficient hemolysis to liberate adequate amounts of pigment, and to hemoglobinuria when the hemolysis is particularly severe and rapid. Unless there is a concomitant depression of the bone marrow activity in addition to the hemolytic process there will be evidences of erythrocytic regeneration and a leukocytosis.

Cold Hemagglutination

The erythrocytes of certain individuals are agglutinated by their own serum when cooled to 0° to 5° C. but are not agglutinated at temperatures above

20° C. The agglutinins in the serum which cause the phenomenon are called "cold agglutinins" and will agglutinate cells of any blood group when brought to the proper temperature. This may be apparent in the counting chamber while doing an erythrocyte count; warming the solution will prevent the reaction. Cold agglutinins are found in high titers in a large percentage of patients with primary atypical pneumonia and have been known to produce a severe hemolytic anemia in this condition and to cause peripheral vascular disturbances similar to Raynaud's syndrome. Their significance in various types of hemolytic anemia is not well understood.

Infections and Parasitic Infestations

A common cause of hemolytic anemia is septicemia due to hemolytic streptococcus or *Streptococcus viridans* and is encountered in puerperal sepsis and some cases of subacute bacterial endocarditis. Certain strains of staphylococcus and other organisms may cause a similar reaction. The method by which these organisms produce hemolysis is unknown; it has not been ascertained whether it is due to the bacteria themselves or to the action of their toxins. Practically all chronic bacterial infections cause some degree of anemia, but in most cases it is due to a toxic inhibition of the bone marrow rather than to actual destruction of erythrocytes.

Hemolytic anemia is occasionally encountered in syphilis and in other spirochetal infections. Oroya fever, an infestation by *Bartonella bacilliformis*, causes a particularly severe anemia which is commonly accompanied by a leukocytosis. The toxins liberated by certain parasites such as the hookworm (*Ancylostoma duodenale* and *Necator americanus*) and the fish tapeworm (*Diphyllobothrium latum*) may cause hemolysis of erythrocytes and so account in part for the anemia which characterizes the infestation.

Malaria

The anemia which accompanies malaria is particularly striking and constitutes one of the important features of the disease. It is a direct result of the destruction of erythrocytes since each cell which harbors a parasite is destroyed. The erythrocyte count may drop significantly after each chill. There is an elevation of the bilirubin content of the blood serum. In addition to the hemolysis caused directly by the parasite it is probable that some destruction of erythrocytes is due to excessive phagocytosis by reticulo-endothelial cells or to a hemolysin. The reduction in the hemoglobin level is proportionate to the lowering of the erythrocyte count so that a normal color index is maintained. There is an increase in the number of reticulocytes

A leukocytosis occurs during a paroxysm of fever and chills, but the white count soon falls and a mild leukopenia develops

The anemia may develop rapidly in severe cases of malaria, especially in those having the estivo-autumnal type. It may be less severe and develop more slowly in those with a milder form. After the disease has been present for a week or more, the erythrocyte count may fall below 2,000,000 cells per cubic millimeter, and there is a tendency for it to stay at this low level until treatment is instituted. In patients in whom the disease is chronic and of long standing the anemia is persistent; a great deal of their weakness and invalidism is attributable to this. Anemia is not a complication but an integral part of the clinical picture produced by malaria. It is frequently augmented by a nutritional anemia brought about by anorexia and inadequate food intake by the patients

The anemia improves after the malaria has been brought under control by adequate therapy. Administration of an inorganic iron salt such as reduced iron (3 Gm. daily) or ferrous sulfate (1 Gm. daily) will hasten recovery, but this is not necessary except in patients with a severe grade of anemia. Liver or liver extract is of no value unless a nutritional anemia is present as a complication.

Blackwater Fever

Blackwater fever is due to a rapid hemolysis of erythrocytes which liberates hemoglobin into the blood stream so rapidly that hemoglobinuria results. This imparts a dark brown or black color to the urine.

The etiology of blackwater fever has not been definitely settled, but it is thought to be a result of repeated attacks of malaria or of a continuous infection. It is most common with the estivo-autumnal variety but may occur with other forms. It is also common in those who have taken quinine irregularly so that the part played by this drug in its etiology is not known. It is believed by Castenada and other physicians who are in the tropics and in contact with the disease continuously that it is due primarily to a malarial infestation of patients whose erythrocytes are in some way peculiarly susceptible to hemolysis.

The onset is sudden with a chill, fever, and pains in the abdomen, back, and legs. The urine which is passed after the onset of symptoms is dark in color owing to the presence of hemoglobin and its derivatives. The manifestations may subside and then recur with each malarial chill or they may persist.

In severe cases the abdominal pain becomes a prominent feature, icterus

appears and gradually deepens, and the urinary output lessens or may be completely suppressed. Uremia, subsequent to anuria, is a common cause of death. The symptoms and pathologic findings are similar to those described in the section on hemoglobinuria except as they are modified by the co-existence of malaria.

Some cases are mild and transient with little or no fever, but one attack appears to predispose to subsequent episodes. A third attack is usually fatal although as many as sixteen attacks have occurred in one individual. The mortality is extremely variable but averages about 25 per cent.

The treatment consists of supportive measures to combat shock, adequate fluid intake to promote diuresis, using particularly intravenous infusions of glucose, and an adequate diet after the acute manifestations have subsided. Because of the liver damage that occurs in these individuals a high protein, high carbohydrate, and low fat diet with a high vitamin content should be given when the patient's condition is such that this diet will be tolerated. During the acute stage reliance must be placed on intravenous fluids and a liquid or soft diet. Antimalarial therapy should be resumed cautiously.

Chemicals and Drugs

There are many chemicals and drugs which produce hemolysis of the erythrocytes. Most commonly encountered or most pronounced in their action are potassium chlorate, acetanilid, nitrobenzol, trinitrotoluene, and other compounds of phenol, benzol, and toluol, arseniureted hydrogen, phenylhydrazine, and arsenic. Severe burns and certain snake venoms may produce a similar reaction. These drugs and chemicals may cause an acute, rapidly fatal hemolysis or a less severe and more chronic intoxication with anemia, jaundice, and increased bilirubinemia.

Sulfonamides

Acute hemolytic anemia is one of the complications which may occur with the administration of sulfanilamide, sulfapyridine, and other of the sulfonamide compounds. In most instances the hemolytic action appears soon after the administration of the drug is begun, usually on the second to the fifth day of treatment, and may be rapidly progressive and fatal. The hemolytic reaction may be preceded by a drug fever or other evidences of drug intoxication or it may appear with no premonitory symptoms. In other instances a high blood concentration of the drug may be carried for many days before hemolysis becomes evident. There is no apparent correlation

between the dosage or the blood level of the sulfonamide and the appearance of the hemolysis so that an individual susceptibility has been presumed, possibly owing to the fact that in certain persons hemolytic products are formed from the drug. Readministration of the drug to these individuals is apt to cause a reappearance of the hemolysis. An increased excretion of urobilinogen in the feces is found in many patients who do not develop clinical evidences of hemolysis. There is no correlation between the amount of urobilinogen excreted and the amount of the drug given. Autoagglutinins are formed in some patients receiving these compounds so that blood typing and cross matching of the blood in preparation for transfusions may become difficult or impossible.

The hemolysis of erythrocytes is associated with a sudden drop in the hemoglobin and erythrocyte values, jaundice, increased urobilinogen in the stools, nausea, vomiting, fever, and exhaustion. Shock may occur in the severe cases. Hemoglobinuria occurs occasionally, and crystals of acid hematin may cause blockage of the renal tubules.

A majority of the patients recover, only a few cases terminate fatally. The treatment consists of withdrawal of the drug and administration of large amounts of fluids and transfusions when necessary.

Lead Poisoning

The hematologic picture in lead poisoning constitutes one of the important diagnostic features of this intoxication and appears to be produced primarily by excessive destruction of erythrocytes. Lead poisoning has been recognized for a great many years. Its various clinical manifestations were correlated as early as 1831. It still represents one of the most common industrial diseases in spite of the fact that recent improvements in preventive measures and in working conditions have greatly reduced its incidence. The respiratory tract is the most frequent as well as the most dangerous portal of entry as the lead quickly enters into the circulation and becomes widely distributed throughout the body. Lead may also be absorbed through the gastrointestinal tract, the mucous membranes of the nose and mouth, and possibly the skin. Following absorption it is carried to all organs of the body but is deposited primarily in the bones. Symptoms of lead intoxication appear when the lead is circulating in the blood stream. This may occur during the period of absorption or during the process of elimination of lead that was previously stored in the bones.

Symptoms. Acute poisoning is relatively rare. It may occur after the ingestion of large amounts of lead or in susceptible persons with only a relatively slight exposure. There is weakness, vomiting, diarrhea, and collapse, and occasionally convulsions occur from the lead encephalopathy. An anemia may develop rapidly as a result of the hemolysis of erythrocytes. This may cause jaundice and occasionally hemoglobinuria.

The chronic type of poisoning is far more common. The symptoms consist of weakness, lassitude, constipation, severe and persistent abdominal cramps, anemia, and peripheral neuritis. The abdominal pain is usually located in the lower quadrants. It may be one of the most distressing manifestations of the illness and is frequently very resistant to therapeutic measures. It is not accompanied by muscle spasm. The peripheral neuritis usually affects the extensor muscles of the wrists and fingers, giving a characteristic wrist and finger drop, but it may also appear in the peroneal muscles of the legs, causing a steppage gait. Optic neuritis may develop, and cerebral manifestations such as depression, delirium, convulsions, or psychoses occasionally appear. The "lead line" on the gums is an important sign of lead intoxication and consists of a deposit of lead sulfide along the margins of the gums. When viewed under a magnifying glass it is seen to consist of multiple small punctate bluish black dots. A similar line may be caused by bismuth.

Hematologic Features. One of the earliest manifestations of chronic lead poisoning is an anemia which is usually moderate in degree and presents a color index slightly below normal. The associated pallor is so striking that it suggests a greater degree of anemia than actually exists. The anemia is apparently caused by an excessive destruction of erythrocytes, which is brought about by the action of lead on the surface membrane of the cell making it more fragile and more easily hemolyzed than normal. One of the most characteristic features of the blood picture is the presence of large numbers of erythrocytes which show a fine basophilic stippling. This stippling is apparent with the ordinary staining methods but is best demonstrated by drying the blood smear in air for twenty-four hours and then staining with dilute Unna's alkaline methylene blue. The reticulocyte count is also elevated. The stippled cells are probably degenerating reticulocytes—all stippled cells are reticulocytes but not all of the reticulocytes are stippled. The presence of stippled erythrocytes is not diagnostic of lead poisoning. They occur in other types of anemia in which blood regeneration is very active. The presence of from 100 to 300 stippled cells per million erythrocytes is significant, however, and constitutes one of the earliest and most

reliable signs of lead poisoning in a person exposed to this metal. The leukocytes may be moderately increased in number.

Diagnosis. The diagnosis of lead poisoning is based on the clinical and hematologic findings already described but should be confirmed by a determination of the amount of lead being excreted. The lead in the feces is of little diagnostic significance since it may have passed through the gastrointestinal tract without being absorbed. The lead found in the urine is significant, however, since it represents lead that has been absorbed and excreted. When urinary excretion is in excess of 0.1 mg. per twenty-four hours, lead poisoning is indicated. Roentgenograms in children who may have acquired lead poisoning from eating paint from their bed or toys may show a line of increased density from a deposit of lead in the epiphyseal ends of the bones.

Treatment. There is no specific therapy for the anemia of lead poisoning, but a prompt improvement will occur when the lead intoxication is relieved. For the treatment of lead poisoning the reader is referred to any of the standard texts on internal medicine.

HEMOLYTIC ANEMIAS OF INTRINSIC ETIOLOGY

The pathogenesis of the hemolytic anemias of the intrinsic type is not the same in all cases. Sometimes the disease is due primarily to a dysfunction of that part of the hematopoietic system which has to do with the formation of erythrocytes so that the circulation is flooded with abnormal cells, and the hemolysis is a compensatory mechanism to rid the blood stream of them. In other instances the excessive hemolysis appears to be the primary feature, and the compensatory hyperplasia of the hematopoietic system is an attempt to replace the abnormally large number of erythrocytes which have been destroyed. The presence of immature cells is one result of the compensatory hyperactivity. The bone marrow in these diseases is functionally intact and capable of forming erythrocytes in adequate numbers. With the continuous destruction of the cells, there is a plentiful supply of the materials necessary for new cell formation and there is some experimental evidence to suggest that products derived from the destruction of erythrocytes may act as a stimulus to new cell production.

Certain hematologic features are common in varying degrees to all types of hemolytic anemias: (1) Anemia. This is usually of a normochromic or slightly hyperchromic type and will vary in severity from one patient to another as well as from time to time in the same patient. It depends upon

the excessive destruction of erythrocytes, but its severity is modified by the rapidity with which new cells are formed. A rapid regeneration of cells may compensate for the excessive hemolysis so that the resultant anemia will be of only moderate severity. (2) Jaundice. This results from destruction of erythrocytes and the consequent liberation of excessive amounts of pigment derived from the hemoglobin. The increased bilirubinemia is demonstrable by chemical tests on the blood serum. These reveal an elevated icterus index and a positive indirect van den Bergh reaction. The urobilinogen excretion in the feces is greatly increased and the urobilinogen content of the urine may be increased, although the latter apparently depends to a great extent upon associated liver insufficiency rather than on excessive hemolysis alone. (3) Reticulocytosis. There is hyperplasia of the erythropoietic tissues, which results in the liberation of an unusually large number of reticulocytes into the blood stream. It is a manifestation of the rapid production of cells. In addition to the reticulocytes there are varying numbers of polychromatophilic and nucleated erythrocytes in the peripheral blood stream. (4) Leukocytosis. As a result of the hyperplasia of the bone marrow production of leukocytes is increased and the percentage of nonsegmented or band neutrophils is higher. Myelocytes and metamyelocytes may appear in the blood stream in varying numbers in cases in which this reaction is severe. These have occasionally been so numerous as to suggest leukemia, and the term *pseudoleukemia* is erroneously applied to this condition. The latter two features, immature erythrocytes and extreme leukocytosis, are particularly prominent when a severe form of the disease appears in infants and children, possibly because the hematopoietic system at this age is more labile and responds with greater intensity to a given stimulus than does that of an adult.

An attempt has been made to subdivide the primary hemolytic anemias into a number of separate clinical entities. Such classification is difficult in many instances, particularly when the manifestations of the disease are slightly atypical. The barriers which have been erected to delimit the various entities are arbitrary and artificial. Many of them will undoubtedly be altered or removed entirely as our knowledge of the diseases increases. Hemolytic icterus has been subdivided into the congenital and acquired types, and from the latter group an acute febrile form has been separated. These conditions have so many features in common, however, and the points of differentiation are so meager, that their logical subdivision into separate clinical entities is open to question. For the sake of clarity and for simplicity in presentation this somewhat arbitrary classification will be retained.

FAMILIAL HEMOLYTIC ICTERUS
CONGENITAL (FAMILIAL) HEMOLYTIC ANEMIA (ICTERUS),
CHRONIC ACHOLURIC JAUNDICE

Familial hemolytic icterus is a chronic hereditary and congenital anemia characterized by recurring episodes of hemolysis with jaundice, increased fragility of the erythrocytes, and splenomegaly. It is transmitted as a dominant mendelian characteristic by either sex but is not transmitted by members of the family who do not have the disease. In many instances the in-

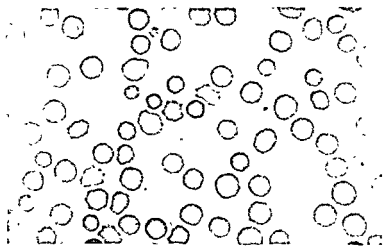


FIG. 35. Photomicrograph of erythrocytes from a patient with familial hemolytic icterus. Note the predominance of small, deeply stained cells and the absence of the normal light staining area in the center. This indicates that the cells are unusually thick and have lost their normal biconcave configuration.

herited defect in the erythrocytes has been present throughout life, but hemolysis has been so slight that it has not produced clinical manifestations. The disease may be transmitted by these individuals to their offspring. Under such circumstances a family history of the disease may be difficult to obtain, but examination of the blood of the parents and siblings of the patient may bring to light an increased fragility of the erythrocytes in some member or members of the family. Questioning may elicit a history of mild recurrent icterus in one of the parents.

Pathogenesis

The two most characteristic features of congenital (familial) hemolytic icterus are (1) increased fragility of the erythrocytes and (2) spherocytosis. Examination of the blood smear (Fig. 35) reveals that the average

diameter of the erythrocytes is less than normal and that those cells which appear to be microcytic actually stain more deeply than normal. When the cells are examined in a hanging drop or other type of moist preparation, it is found that although their diameter is decreased, their thickness is increased. Determination of the mean corpuscular volume reveals that the actual volume of the cell is normal or increased. The greater thickness of the cell has compensated for its smaller diameter, and it has lost its normal biconcave configuration to become thicker and more nearly spherical in shape. Cells of this type are called spherocytes.

This change in the structure of the erythrocyte was noted by Chauffard. Its effect on the fragility of the cell was particularly stressed by Haden. When normal erythrocytes are placed in a hypotonic saline solution, fluid passes into the cell because of the difference in osmotic pressure. This produces swelling, an increased thickness of the cell, and ultimate rupture of the cell membrane with hemolysis. Hemolysis is first apparent with normal cells in a 0.44% solution of saline and is complete—all the cells hemolyzed—in 0.34 % saline. The thickness of a spherocyte is already greater than normal so it is incapable of taking in as much fluid without rupture of its membrane as is the normal biconcave erythrocyte. The cell will therefore hemolyze more readily when exposed to hypotonic solution (Fig. 36). The presence of large numbers of spherocytes in the blood stream is the accepted explanation for the increased fragility in hemolytic icterus and accounts for the fact that the blood may show beginning hemolysis in 0.7 to 0.8% saline and complete hemolysis at a point correspondingly higher than the normal.

There are different opinions as to the cause of the spherocytosis. Any consideration of this subject must take into account the fact that splenectomy produces a clinical cure although the spherocytosis and increased fragility remain unchanged or are but slightly altered. The accepted explanation is that these abnormally fragile cells are produced in the bone marrow and are abnormal at the time they enter the blood stream. The spleen, one of the functions of which is to remove abnormal and degenerated erythrocytes from the circulation, is unusually active in destroying the abnormal cells, and an excessive hemolysis results. Splenectomy does not alter the production of spherocytes but removes the organ which is most actively engaged in their destruction so that the clinical evidences of increased hemolysis are removed even though the abnormal cells persist in the circulation.

A second theory holds that the cells are originally normal in shape and in osmotic resistance but are altered in these respects after they enter the blood stream. This concept proposes that the erythrocytes are exposed to hemo-

lytic agents or hemolysins of some type within the blood stream and that the spherocytosis and increased fragility are early stages in the hemolytic process. Hemolysins of the immune body type have been demonstrated by Dameshek in some, but not all, cases of acquired hemolytic icterus, and spherocytes have been produced in experimental animals by the injection of hemolytic serums. Autoagglutinins which produce clumping of the eryth-

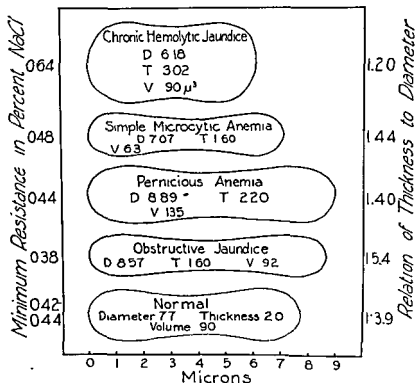


FIG 36 Diagram showing the cross section configuration of erythrocytes in various diseases as compared to the normal and the effect of their shape on the resistance to hypotonic saline solution D = mean diameter in microns, T = thickness, V = corpuscular volume. (Haden, *Am J M Sc*)

rocytes on smears, in cross matching, and when erythrocyte counts are taken have been encountered in some cases, but neither these nor autohemolysins can always be demonstrated, and their significance is still undetermined.

A hemolyzing substance, lysolecithin, has been found in normal blood and can be increased in amount by incubating blood (without shaking) in vitro. The spleen is a reservoir which contains an enormous volume of blood in hemolytic icterus. It is possible that lysolecithin is developed to higher than normal concentrations or that it is produced in increased amounts in

patients with this disease. Spherocytosis under these circumstances would be an early stage in the process of hemolysis. Inability to consistently demonstrate an increased amount of lysolecithin in the blood of patients with hemolytic icterus has prevented the acceptance of this theory.

It has been demonstrated that intravascular stasis, as well as sterile incubation of blood in vitro, will produce spherocytosis and increased fragility of the erythrocytes to the extent that they will be hemolyzed in isotonic saline. It has been suggested that the stasis which occurs in the greatly enlarged spleen of patients with hemolytic anemia may account for the increased fragility in those patients in whom hemolysins or hemolytic agents are not present.

A final opinion regarding the cause of spherocytosis and the increased rate of hemolysis cannot be given at present although recent investigative work in this field gives promise of enlightening information.

Pathology

The tissues from patients with congenital hemolytic icterus are stained with bile pigments, the extent of the staining varying with the severity of the hemolytic process. There are deposits of iron-containing pigment, sometimes very extensive, in all of the organs. The spleen is markedly enlarged and has a smooth glistening surface and a deep bluish red color. It may be 4 to 10 times normal size. On cut section it is deep red in color and presents a picture of intense congestion which, on microscopic examination, is found to consist of a marked engorgement of the pulp by erythrocytes with relatively empty blood sinuses. The lymph follicles are small and atrophic and the trabeculae are indistinct. Infarctions are frequently encountered. The liver is enlarged to a moderate degree and may show fatty degeneration and deposits of iron-containing pigment. As a rule there are no other significant alterations although an increase in the size and number of the Kupffer cells has been reported in some instances. Gallstones are found in the biliary tract in a large percentage of the cases.

The hematopoietic bone marrow is hyperplastic and replaces the fatty marrow to varying degrees. Meulengracht records an instance in which the entire shaft of the femur was filled with hematopoietic marrow. Microscopic examination reveals that nucleated erythrocytes predominate in the hyperplasia. The leukopoietic elements are involved to varying degrees, but in some instances both myeloid foci and megakaryocytes are scarce and apparently crowded out by the erythropoietic elements. Extramedullary hematopoiesis has been observed, usually in severe cases occurring in infants.

Clinical Manifestations

The manifestations and the age at which this disease makes its appearance are variable. There are latent cases which show the specific defect in the erythrocytes but few or no clinical manifestations. The first indication of the disease in these cases is its appearance in the patient's offspring. In other instances there are recurring episodes of slight jaundice which are never severe enough to cause significant disability. In severe cases there are hemolytic episodes during infancy or childhood resulting in chronic invalidism with recurring acute exacerbations. In patients presenting the more severe clinical manifestations the disease usually becomes apparent in infancy or childhood. As a rule, the later in life the manifestations appear, the milder will be the course. In the typical case the patient feels well or has only mild symptoms and a slight degree of icterus most of the time. At irregular intervals there occur exacerbations with increasing weakness, fatigability, shortness of breath, pallor, and jaundice. These recurring episodes of increased hemolysis or "crises of deglobulization" constitute one of the most characteristic clinical features of the disease. They may occur at any time with no apparent cause but are frequently initiated by infections, exhaustion, or trauma. After persisting for a variable length of time the acute episode spontaneously subsides, and the condition reverts to the quiescent stage.

Hemolytic episodes are more frequent and more severe in some cases and are associated with fever, prostration, nausea, vomiting, and at times severe abdominal pain which may be in either the right or the left upper quadrant. The episodes of pain are a part of the picture of active hemolysis, but attacks of true biliary colic are also frequent in these patients. An unusually large amount of bile pigment is formed because of the liberation of excessive amounts of blood pigment. When this hemolysis has been occurring over a long period of time, there is great tendency toward the formation of gallstones. These are frequently encountered in young children so that biliary colic and obstructive jaundice may be superimposed on the picture of hemolytic icterus at any age. It must be remembered, however, that the hemolytic episode itself may cause abdominal pain, when this is accompanied by fever and leukocytosis, it may suggest an acute condition of the abdomen in which surgical intervention is indicated. The jaundice of hemolytic icterus is ordinarily not as intense as that associated with obstruction of the biliary tract, and acholic or clay-colored stools are not encountered unless the disease is complicated by such obstruction. Hemolytic jaundice is not associated with pruritus, nor are bile pigments to be found

in the urine. Jaundice of a slight degree is almost constantly present in patients with this disease, and, except during the acute episodes and in the more severe cases, the statement that the patient is "more jaundiced than ill" holds true.

The degree of pallor varies with the severity of the anemia, which may be mild or severe. The spleen is consistently enlarged and may become a massive organ causing a sense of weight or a dragging sensation in the left upper quadrant of the abdomen. This is occasionally one of the chief complaints. There is a tendency for the spleen to increase in size with the acute hemolytic episodes and to recede during remissions. Pain in the splenic area is a common symptom and may be due to the perisplenitis which accompanies infarction of the organ. The liver is enlarged in about one-third of the patients but usually not excessively. There may be a slight degree of cardiac enlargement due to dilatation, and a hemic murmur may be heard.

Chronic ulcerations on the lower leg, similar to those so frequently found in sickle cell anemia, are occasionally encountered in familial hemolytic icterus. These ulcers are rounded and sharply demarcated or "punched out" in appearance; on healing they leave a smooth glossy scar or a deeply pigmented area. Although healing may occur during a spontaneous remission, in the more severe cases the ulcerations may persist until splenectomy has induced a complete clinical remission.

Röntgen Ray

Roentgenologic examination of the bones may reveal significant findings in children with a severe form of the disease. The changes are due to hyperplasia of the marrow, which causes decalcification of the trabeculae, an increased volume of the medullary portion of the bone, and a consequent thinning of the cortex. This results in a generalized mottling or spongy appearance of the bones of the skull with thickening of the medullary portion and a thinning of the outer and inner tables. New bone formation may occur as perpendicular striations surrounding the outer table. The generalized decalcification is especially evident in the hands and feet. The roentgenologic changes are the same as those which are more completely described in the section on erythroblastic anemia. A "tower skull" is one of the developmental abnormalities of the skeletal system which may be encountered.

Laboratory Findings

The hematologic findings vary with the severity of the disease and the acuteness of the hemolytic process at the time of examination. During a

quiescent period the erythrocyte count and hemoglobin level may be normal or but slightly lowered although the more severe cases have a persistent anemia of varying degree. The reduction in the erythrocyte count and in the hemoglobin level is proportionate so that the color index remains about normal. During the hemolytic crises there is a fall in the red cell count and hemoglobin level which may be rapid and profound. Erythrocyte counts of 1,000,000 or less are occasionally encountered in severe cases. The erythrocytes and hemoglobin persist at these levels for a variable time and then spontaneously increase as the hemolytic activity subsides. In other instances the exacerbations are mild so that even during the acute stage of the disease the anemia may be of only moderate degree.

The increased bilirubin content of the blood is commensurate with the severity of the hemolytic process. The icterus index is high, and the van den Bergh test is positive with an indirect reaction. The urobilinogen content of the feces is greatly elevated, and the urobilinogen output in the urine may be increased. Watson has suggested that the increased urinary urobilinogen is due to liver insufficiency rather than to the liberation of excessive amounts of blood pigment alone. When obstructive jaundice intervenes, bile will not be present in the feces and bile pigments will be found in the urine.

On examination of the smear the evidences of spherocytosis will be noted, the erythrocytes appearing small but more deeply staining than normal. The apparent microcytosis is misleading, however, since the thickness of the cells has increased sufficiently to compensate for their decrease in diameter. Their actual volume is normal or increased. They are no longer biconcave disks but have become more nearly spherical in shape (Fig. 37).

As was previously mentioned, these cells have a diminished osmotic resistance as measured by exposing them to varying concentrations of hypotonic saline solution. Their increased fragility is congenital and is constantly present throughout life but becomes more marked during the acute episodes and does not disappear even after a clinical cure is brought about by splenectomy. This altered resistance of the erythrocytes is one of the most important diagnostic features of the disease and will be found in 90 per cent of the cases.

The reticulocytes are more numerous, particularly during an acute hemolytic episode. A spontaneous reticulocytosis of 90 per cent has been observed, and readings of from 40 to 50 per cent are not at all uncommon. There is a variable degree of polychromatophilia, and a few nucleated erythrocytes are frequently encountered. These are more numerous in younger patients with a severe form of the disease.

A leukocytosis of variable degree is common during the acute episodes. The count returns to normal during quiescent periods. Normal or even lowered leukocyte counts have been recorded during acute episodes. Leukocytosis is more frequently encountered in young patients than in adults and is usually due to an increase in neutrophils. There is an increased percentage of nonsegmented or band forms, and varying numbers of metamyelocytes and myelocytes frequently appear, sometimes to the extent that myeloid



FIG. 37. Bas-relief photomicrograph showing the increased thickness of the cells leukemia is simulated. The platelets may be increased but usually are not significantly affected.

The bone marrow shows a normoblastic type of hyperplasia with marked evidences of increased erythrocytic production as might be expected from the reticulocytosis and polychromasia of the peripheral blood.

Diagnosis

The diagnosis of familial hemolytic icterus is based upon the jaundice, anemia, reticulocytosis, and leukocytosis, which are the characteristic features of all hemolytic anemias, plus the increased fragility of the erythrocytes, spherocytosis, splenomegaly, and the familial history. The greatest difficulty in differential diagnosis in children is presented by the erythroblastoses and Cooley's anemia. In neither of these is there an altered fragility of the erythrocytes, spherocytes are not present, and the number of nucleated erythrocytes is usually much greater. There may be a familial trait in the erythroblastoses, and roentgenologic changes which are similar to those found in erythro-

blastic anemia may occasionally be encountered in the bones of infants with hemolytic icterus.

Hemolytic icterus in an adult may be confused with pernicious anemia, but the macrocytosis, high color index, leukopenia, and normal fragility of the erythrocytes should distinguish the latter condition. Neurologic changes do not occur in familial hemolytic icterus, nor are paresthesias encountered.

Acquired hemolytic icterus presents a picture similar to the familial form, but there is an absence of the typical family history. The other minor differentiating points are considered in the discussion of that disease. The presence of jaundice and hyperbilirubinemia should easily distinguish hemolytic icterus from aplastic anemia and the iron deficiency anemias. Banti's syndrome or splenic anemia may present more difficulty because of the enlarged spleen and icterus, but the leukopenia, the absence of an increased fragility, and the negative family history should serve for differentiation.

Prognosis

There is a great variation in the severity of the disease. Many cases are latent or extremely mild so that little disability results although all such patients are liable to have acute and severe exacerbations at any time. In other patients the acute hemolytic episodes are frequent and more severe; the patient may become a chronic invalid. Death may occur with a hemolytic crisis. When the disease appears early in life, its course is usually more severe than when it appears in adults.

Treatment

Splenectomy is the specific form of treatment for congenital hemolytic icterus. This procedure must be given due consideration in every case in which the diagnosis is made. The severity of the symptoms and the age of the patient should, however, be taken into consideration before advising the operation. Splenectomy is not indicated in the very mild or latent forms that first make their appearance in older adults. The disability may not be severe enough to prevent an active and useful life in these patients, and it is probable that the manifestations will become even less severe with advancing age. However, an acute episode of hemolysis may occur at any time even in mild and heretofore quiescent cases. A close check for evidences of increased hemolysis should be kept on the patients on whom splenectomy is not performed. When the symptoms of the disease make their appearance in a child or young adult splenectomy should be performed even though the disease has produced only a mild degree of ill health. The spleen should be removed

during a remission if possible rather than during an episode of acute hemolysis. It is impossible to wait for the opportune time in some instances, and the surgical procedure must be carried out at once.

Splenectomy results in complete relief of the clinical manifestations in practically all cases, but the increased fragility of the erythrocytes and the spherocytosis persist even in the absence of other manifestations. Following the operation there is an immediate decrease in the rate of hemolysis as is shown by a lessening in the degree of jaundice, a drop in the bilirubinemia, and a decrease in the fecal output of urobilinogen. The anemia improves without other therapeutic measures. A striking increase in the number of erythrocytes may be noted within a few hours after the operation. This immediate response is due in part to the emptying of the splenic reservoir into the peripheral blood stream during the operative manipulations. Compression or "milking" of the spleen is an advisable procedure prior to ligation of the vessels. The disease is still transmissible by a parent in whom splenectomy has resulted in complete relief of the subjective manifestations.

Gallstones are present in a high percentage of patients with familial hemolytic icterus and have frequently been encountered in children below the age of 10. When symptoms due to biliary calculi are present, the question frequently arises as to the correct sequence of surgical procedures—that is, whether to first perform a cholecystectomy to relieve the biliary obstruction and remove the spleen at a later date or to proceed at once with splenectomy. To carry out both procedures at one time is usually inadvisable. It has been found that trauma, such as occurs with a laparotomy, may initiate a severe and sometimes fatal hemolytic crisis. For this reason splenectomy should be performed first, except in the few cases when it is imperative that biliary obstruction be relieved immediately.

Transfusions are of only temporary benefit and are seldom indicated in familial hemolytic icterus. In most cases the anemia is not severe enough to require them, but in the more acute forms they may be necessary as a preoperative measure. Attention has been called to the danger of hemolytic reactions following transfusions. These not only may lead to hemolysis of the transfused cells but may initiate or accentuate the hemolysis of the patient's own cells and so aggravate the already serious condition of the patient. Such acute hemolytic reactions may lead to blocking of the kidney tubules by deposits of acid hematin crystals. Anuria and death from uremia may result. When a transfusion is imperative, it is best to use a donor of the same blood group rather than to rely on a universal donor and to cross match the cells and serum of both donor and recipient. The recipient's serum should also be

tested for a hemolytic action on the donor's cells. If the patient is so anemic as to require transfusion, a splenectomy should be done as soon as conditions will permit since it frequently produces a striking and immediate improvement.

Better results are obtained when splenectomy is performed early in the course of the disease as this checks the formation of biliary calculi. TempORIZING with other therapeutic measures is not justifiable except in very mild or latent cases. The operative mortality from splenectomy is in the neighborhood of 4 or 5 per cent.

The administration of iron and liver extract does not benefit this type of anemia. Doan has reported that liver extract is not only valueless but may initiate a hemolytic crisis

ACQUIRED HEMOLYTIC ICTERUS

Although congenital or familial (Chauffard-Minkowski) hemolytic icterus must be considered as a distinct clinical entity, there is a difference of opinion as to whether the acquired (Hayem-Widal) type should be so recognized. Many feel that the term *acquired hemolytic icterus* should be discarded. They believe that all such cases are really manifestations of the familial form and that there is a latent, inherent defect in the erythrocytes of these individuals which permits easy hemolysis and requires only some infectious, toxic, or metabolic disturbance to make it apparent. A diagnosis of acquired hemolytic icterus has been made in many cases only to find on further investigation that mild and heretofore unrecognized symptoms of the disease have been present in other members of the family or to have siblings who previously had shown no evidence of the disease subsequently develop typical hemolytic icterus. Most cases of primary hemolytic icterus are undoubtedly of the familial or congenital type.

The term *acquired hemolytic jaundice* was applied by Widal to a group of cases which were apparently not congenital or familial and which appeared either in the course of various illnesses or in a primary idiopathic form. He recognized the absence of a hereditary factor, the moderate alteration in the fragility of the cells, and the autoagglutination of the erythrocytes. Many subsequent reports on this disease have included cases which were apparently atypical examples of pernicious anemia, Banti's syndrome, and other conditions. There remain, however, some cases which seem to justify the term *acquired hemolytic icterus*. This group has been further subdivided into (1) those which are idiopathic or primary in origin and (2)

those which are secondary to some other disease. In its secondary form the disease has been found associated with syphilis, malaria, tuberculosis, septicemia, and other infections, as well as with leukemia, Hodgkin's disease, cirrhosis of the liver, and pregnancy. Eradication of the primary disease will frequently effect a cure of the hemolytic process. The course in those cases which we have observed in association with pregnancy has been unusually mild. The manifestations have disappeared following delivery although there is a tendency for a recurrence with succeeding pregnancies. In one patient a typical hemolytic episode occurred during a pregnancy and a subsequent episode during the course of an infection.

In the idiopathic form of acquired hemolytic icterus, which closely simulates the familial form, there is no recognizable causative factor. Questions concerning its pathogenesis have been considered in connection with familial hemolytic icterus. Autoagglutination of the erythrocytes has been frequently noted in doing erythrocyte counts, in cross matching or typing the blood, or by resuspending the cells in the patient's own serum. Isohemolysins have been found by Dameshek in some cases. It is possible that a mechanism of this type may be responsible for acquired hemolytic icterus.

Clinical Features

The clinical manifestations of the idiopathic type of acquired hemolytic icterus are similar to those of the congenital form except for minor variations. The disease usually makes its appearance during early or middle adult life but is occasionally found in children. The onset may be gradual with a progressive anemia or sudden with fever, prostration, abdominal pain, and a rapidly developing jaundice and anemia. A rapid onset is more frequent in the acquired than in the congenital form, and the hemolytic crises are more frequent and more severe. The degree of anemia varies, but a severe grade is commonly encountered. The anemia and pallor are more outstanding than is the jaundice.

The hematologic features are similar to those of the congenital type although spherocytosis and increased fragility of the erythrocytes are less common or are present to a lesser degree. The reticulocyte count is elevated to a variable extent and may be just as high as in the congenital type. A macrocytosis, rather than a microcytosis, is more frequently present. The leukocytic response is similar in the two forms.

The pathologic features are essentially the same in the two conditions. An enlarged and congested spleen is a characteristic finding. On histologic examination it shows increased fibrosis. The hyperplasia of the bone marrow is

the same in both forms as are the evidences of jaundice and the deposits of iron-containing pigment.

Treatment

Splenectomy should be recommended in those cases of acquired hemolytic icterus which are of idiopathic origin and which do not respond to conservative measures. The best results are to be expected in those cases that closely resemble the congenital type in their hematologic features but one cannot recommend the procedure with the same confidence as in the congenital form. The operation is contraindicated in those cases secondary to infections or other diseases.

Liver extract and iron are of no value and transfusions must be given with the greatest caution. Difficulty may be met in cross matching the blood of prospective donors because of autoagglutination of the red cells. In one of our patients it was impossible to obtain a donor whose blood was compatible; consequently, transfusion was impossible. A severe or even fatal transfusion reaction will sometimes occur even though the donor's blood is apparently perfectly compatible with the patient's. This danger appears to be greater in acquired than in congenital hemolytic icterus so that transfusions should be given with the greatest caution. We have observed one fatal hemolytic reaction following transfusion and several mild reactions although some patients have received transfusions with no reaction whatsoever.

ACUTE HEMOLYTIC ANEMIA (LEDERER'S)

The acute hemolytic anemia of Lederer or Brill is characterized by sudden onset of a severe, rapidly progressing anemia of the hemolytic type, associated with high fever, chills, malaise, and jaundice. It may occur in either sex and at any age but is more common in young persons, particularly in the first two decades of life.

Etiology and Pathogenesis

A definite etiologic agent has not been discovered in this type of hemolytic anemia. The manifestations are so similar to those of acquired hemolytic icterus that it may represent merely an acute hemolytic episode in the course of that disease. Dameshek and others have found autoagglutinins and auto-hemolysins in the blood serum of patients with this type of anemia but no correlation existed between the titer of the hemolysin and the degree of hemolysis.

The sudden onset of the disease and the fever suggest that it may result from some infection. However, repeated blood cultures are found to be sterile, and no definite evidences of infection have been found on physical examination or at necropsy. It seems more logical that infection is the initiating or exciting factor rather than the basic cause. It is probable that the hemolysis is due either to some hemolytic agent, possibly extrinsic, or to the presence of isohemolysins in the serum. The bone marrow is able to respond to the excessive demands placed upon it, and when recovery occurs it is apparently complete and permanent so that it is difficult to place the blame for the disease on a faulty hematopoietic function. Because of the excellent therapeutic results obtained with blood transfusion, it has been suggested that a neutralizing substance or antihemolytic agent is introduced with the transfused blood.

Pathology

The pathologic features are not specific. The spleen is enlarged and may show sterile infarcts. There is acute parenchymatous degeneration of the liver with some areas of central necrosis. Excessive amounts of iron-containing pigment are found in all organs as a result of the extensive hemolysis. The bone marrow is red and hyperplastic.

Clinical Features

The onset is usually abrupt without a previous history of anemia or jaundice and is characterized by a rapidly progressing weakness, restlessness, malaise, pallor, and jaundice. These may be accompanied or preceded by gastrointestinal symptoms with diarrhea, anorexia, nausea, vomiting, and severe abdominal pain. The temperature is elevated, commonly reaching 104 F. In the fatal cases a terminal hyperpyrexia is the rule. The usual symptoms of anemia will be noted, and there may be epistaxis. Hemoglobinuria and anuria occasionally occur in the severe cases.

Examination reveals pallor and jaundice, the severity of which depends upon the rapidity of blood destruction. Pallor is usually a more outstanding feature than icterus. The spleen is commonly enlarged, and a slight enlargement of the liver and lymph nodes has been noted in some cases. Retinal hemorrhages and purpura may be present.

Hematologic Features

The anemia progresses rapidly. In severe cases the red cell count may fall to 1,000,000 or less within a short period of time. The color and volume

indices usually remain about normal but occasionally are elevated, and a macrocytosis is evident on the smear in some cases. There is a reticulocytosis; polychromatophilia and nucleated erythrocytes are frequently encountered. The leukocytes are usually more numerous, and counts as high as 50,000 and over have been found although in some of the reported cases there has been a normal or low leukocyte count. In the blood of those patients with a marked leukocytosis there are evidences of immaturity of the myeloid cells with the occasional appearance of a few myelocytes and metamyelocytes. The platelets are not affected. There is no alteration in the fragility of the erythrocytes—an important feature in differentiating the disease from an acute exacerbation of familial hemolytic icterus. The bilirubin content of the blood is increased, and the urobilinogen excretion in the feces is markedly elevated. The amount of urobilinogen in the urine may increase but does not parallel the rate of hemolysis. When hemolysis is especially rapid, hemoglobin may appear in the urine, which, under certain circumstances, leads to pigment infarcts in the kidneys with subsequent anuria and uremia.

The course of the disease is usually acute so that recovery or death ordinarily occurs within six weeks. The course is chronic in only a very few cases. The temperature drops by lysis during the recovery period, but in cases that terminate fatally there is an extremely high temperature with generalized edema, stupor, and coma. When recovery occurs, it is usually complete with no recurrences or sequelae.

Treatment

Transfusion produces a very dramatic recovery in some cases and the procedure has been looked upon by some as a specific form of treatment. The temperature may fall rapidly and the patient recover completely after a single transfusion. In most cases, however, repeated transfusions are necessary. This mode of therapy is not always successful and death may occur in spite of transfusions, especially if they are not given early in the course of the disease. Splenectomy should be advised in those cases which do not respond to transfusions. It should be performed as soon as it is evident that the transfusions are of no value rather than postponing it as a measure of last resort when the patient is so weak and exhausted that he is a poor operative risk.

There is no apparent benefit from the administration of liver and iron.

SICKLE CELL ANEMIA

Sickle cell anemia is a form of hemolytic anemia which occurs almost exclusively in Negroes. It is characterized by the presence of great numbers of

crescent- or sickle-shaped erythrocytes in the blood stream. The name *menisocytosis* has been applied to the disease.

The condition was first described by Herrick in 1910. All of the early reports suggested that it was found exclusively in Negroes, but a few apparently authentic cases have subsequently been recorded in patients with no admixture of Negro blood. Such cases are exceedingly rare, and the racial incidence is one of the most striking characteristics of the disease. Further studies on patients with sickle cell anemia and on members of their families brought to light the fact that the disease has a definite familial incidence but that only a small percentage of individuals showing the sickle cell trait become anemic. The term *sicklemia* has been applied to that condition in which the typical structural changes are present in the erythrocytes of a person in whom there is no evidence of anemia or history of having been anemic at any time. Sicklemia or the sickle cell trait must be differentiated from "latent" sickle cell anemia—the condition of patients who are in a remission or a quiescent period of sickle cell anemia following one or more episodes of hemolysis.

Sicklemia, the sickle cell trait without clinical manifestations, is a familial, hereditary affliction. It appears to be transmitted as a dominant mendelian characteristic which is not sex linked. It is found in both sexes and has been noted at all ages from birth to the age of 78 years. From the combined statistics of many observers sicklemia has been found to be present in approximately 7 per cent of North American Negroes, but only 1 out of 15 of those so afflicted develops sickle cell anemia.

The anemia which is manifested by a peculiar configuration of the erythrocytes. Attempts to explain the structural changes on the basis either of some substance in the blood serum or of an abnormal action of the spleen have not been convincing.

One of the most striking features in the tissues of patients with sickle cell anemia is the presence of abnormal erythrocytes. It seems, therefore, that phagocytosis of abnormal erythrocytes is the principal cause for the anemia. But the mechanism whereby an acute exacerbation of hemolysis is initiated has not been explained. Infections, toxins, and environmental factors have been considered as possible explanations.

The pathologic picture varies with the stage of the disease. In sicklemia there are no significant pathologic changes in the spleen. In latent sickle cell anemia the splenic pathologic condition is similar to that found in the active

form. This, in turn, varies with the stage of the disease. Diggs has shown that early in the course of the disease the spleen is enlarged, congested, dark purple in color, and soft. Infarcts are commonly present. In the later stages the changes are variable but ultimately result in a spleen which is small, firm, atrophic, and fibrotic. Small nodules containing a yellowish brown pigment and calcium salts are common. The normal structure of the spleen completely disappears as the fibrosis and atrophy increase until the organ becomes a small fibrotic mass which is sometimes buried in dense fibrous adhesions. Its size and the degree of fibrosis vary with the duration of the disease and with the number and severity of the acute exacerbations that have occurred. The liver is enlarged and congested, and there are evidences of increased phagocytosis of erythrocytes. Both liver and kidneys show deposits of hemosiderin. The bone marrow is hyperplastic with red marrow replacing the fatty marrow in some areas and actually invading the cortex of the bone in places. The degree of hyperplasia of the hematopoietic marrow varies with the stage of the disease but may be extreme. In some instances the cortex of the bone is thickened and sclerotic but shows areas of medullary invasion and of necrosis which give the roentgenologic appearance of osteoporosis.

Clinical Features

The abnormal erythrocytes of sickle cell anemia are present at birth, but the onset of symptoms may be delayed for a variable period. It is primarily a disease of children, the average age at which the symptoms appear is 13 years. As a general rule the later in life it makes its appearance, the milder will be the course. It is characterized by alternating periods of excessive hemolysis of the erythrocytes and quiescent or latent periods. During latent periods adult patients have few or no symptoms, but as a rule younger patients are chronically ill, fatigue easily, and are in a poor nutritional state. Moderate grades of anemia and icterus persist. The usual symptoms during a relapse are those which are referable to the anemia: weakness, fatigability, shortness of breath, and palpitation. As the disease becomes more active, the jaundice deepens because of the increased rate of hemolysis, the spleen enlarges, the anemia becomes more severe, and weakness and fatigability become more pronounced. Acute pain in the upper abdomen may be present during the active stage. When this is accompanied by nausea and vomiting, a condition of the abdomen indicating surgical intervention is simulated. Fever of variable height is commonly associated with the acute episodes. Severe aches and pains in the joints and muscles of the extremities are often noted and have led to a mistaken diagnosis of rheumatic fever. The active stage may last

for several weeks or months. Following this there is a spontaneous remission to a more quiescent stage of chronic ill health or a latent stage of variable length in which there are few or no symptoms. The acute attacks are apt to be more severe in children. If the patient survives to adult age, the episodes tend to become less severe and less frequent.

The physical findings differ with the acuteness and duration of the disease. The skin and scleras show a variable degree of icterus during the acute phase which becomes less prominent as the hemolytic episode subsides. It may almost completely disappear during the latent periods. There is pallor of the skin and mucous membranes varying with the severity of the anemia. The spleen is enlarged in younger patients and those in whom the disease is of short duration. It will change in size from time to time depending upon the acuity of the disease, increasing with the acute exacerbations and becoming smaller during the latent periods. When the disease has been present for many years, the spleen is no longer palpable, having shrunk to become small and fibrotic. The liver is slightly enlarged. There may be a slight generalized lymphadenopathy. Chronic ulcers in the region of the ankle are very common in older patients in whom the disease is of long standing. The ulcers may be single or multiple and are round, sharply demarcated with a "punched-out" appearance, and leave a smooth glossy scar on healing. Wintrobe has pointed out that the cardiac complications in sickle cell anemia are particularly severe and that cardiac enlargement, overactive precordia, and various types of murmurs are frequently encountered. These features are at times very suggestive of rheumatic heart disease.

The central nervous system is frequently involved, producing a wide variety of symptoms and physical signs attributable to cerebral or meningeal involvement. These are due to thrombosis of various vessels and may cause headache, drowsiness, hemiplegia, aphasia, stiffness of the neck, and convulsions.

Roentgenologic examination frequently reveals various types of lesions in the bones. Occasionally there is widespread osteoporosis; in other instances there is a thickening of the outer and inner tables of the skull with perpendicular striations radiating outward.

Sternal aspiration reveals a hyperplastic marrow with a predominance of normoblasts.

Laboratory Findings

During an acute episode there is a rapid decrease in the erythrocyte count and hemoglobin level. The reductions are about parallel so that the color

index remains about normal or only slightly lowered and the mean corpuscular volume is unaltered. As the hemolysis progresses, the jaundice deepens and the bilirubin content of the blood increases giving an indirect van den Bergh reaction and a higher icterus index. The urobilinogen content of the feces is markedly elevated. During this stage appear evidences of bone marrow stimulation and hyperplasia as shown by the reticulocytosis, the increased number of polychromatophilic cells, and the large numbers of nucleated erythrocytes. There is a neutrophilic leukocytosis which may reach 20,000 or 30,000 with varying degrees of immaturity in the cells. An occasional myelocyte or metamyelocyte may be encountered. The leukocytic response



FIG. 38 Photomicrograph of sickle cells as seen on a stained dry smear. (Courtesy of L. W. Diggs, M.D.)

is apt to be higher in infants and children than in adults. The platelets may be moderately increased in number.

The anemia is less severe or entirely absent in the chronic or latent stage. Leukocyte and platelet counts are normal.

The characteristic finding, and the one on which the diagnosis is based, is the presence of varying numbers of abnormally shaped erythrocytes. The cells assume many bizarre forms, the most common being the sickle, crescent, or fusiform shape. Other cells are irregularly stellate in outline, the cytoplasm appearing to be drawn out into spinelike projections at two or more points. This sickling may be apparent on the stained smear (Fig. 38) but is far more prominent on a moist preparation in which a drop of blood is sealed under a cover slip within a ring of vaseline (Fig. 39). The sickling may be apparent

as soon as the preparations are made but becomes more pronounced when the blood is allowed to stand without contact with oxygen for from two to twenty-four hours. In many instances no sickling whatsoever will be seen on the fresh preparation, and it is necessary to take other measures to accentuate the sickling trait. Certain dyes hasten the phenomenon so that by treating a drop of blood with cresyl blue, as for a reticulocyte stain, the sickling is accentuated. The sickling trait will also be more apparent if the finger is constricted for five minutes before the blood is drawn. Although the erythrocytes are structurally abnormal and are destroyed too rapidly within the body, there is no increased fragility as measured by exposure of



FIG 39 Photomicrograph of sickle cells as seen in a moist preparation (Courtesy of L. W. Diggs, MD)

the cells to hypotonic saline solution. Many of the erythrocytes are normal in their configuration, others show only minor degrees of poikilocytosis. Target cells are encountered rather frequently. Aspiration of bone marrow material from the sternum reveals evidences of hyperplasia with an increased number of nucleated erythrocytes. These are normal in their appearance.

Diagnosis

The diagnosis of sickle cell anemia is based upon the finding of the characteristic sickling deformity of the erythrocytes plus the presence or a history of hemolytic episodes and anemia. The sickle cell trait alone is insufficient for diagnosis. It must be associated with other clinical evidences. Examination of a stained dry smear is usually insufficient to establish the diag-

nosis, and other methods are necessary to bring out the characteristic deformities of the cells.

Other types of hemolytic anemia, such as familial hemolytic icterus and Cooley's anemia, present similar clinical pictures but are not associated with the characteristic deformities of the erythrocytes.

The acute episodes of abdominal pain, fever, leukocytosis, and muscle rigidity may suggest an inflammatory lesion within the abdomen, and rheumatic fever may be simulated in those patients having severe joint and muscle pains accompanied by fever and leukocytosis.

Prognosis

Sickle cell anemia is a fatal disease although it pursues an extremely chronic and long-drawn-out course in many cases. In other instances, especially when it develops in infancy, it may run an acute and rapidly fatal course. Death may result from the acute hemolytic episodes or from intercurrent infections to which these patients are extremely susceptible. Renal insufficiency has been reported as a cause of death as has thrombosis of cerebral vessels.

The sickle cell trait occurring without anemia does not cause any impairment of the patient's health.

Treatment

There is no specific form of therapy that is of value so that treatment must be entirely symptomatic. Transfusions are valuable as a supportive measure in patients with a severe anemia. They are not without danger, however, and may initiate a hemolytic episode.

Splenectomy has been tried but apparently has no effect on the subsequent course of the disease. The administration of iron and of liver extract has given no evidence of being beneficial. Since the disease has a natural tendency toward spontaneous remissions and because it becomes less severe with advancing age, the results of any therapeutic measure must be evaluated very critically. The patient's general health and particularly the nutritional state should be carefully watched. Infections should be carefully avoided.

ERYTHROBLASTIC ANEMIA (COOLEY'S)

The erythroblastic anemia of Cooley appears early in childhood and has as its most characteristic feature the presence of large numbers of nucleated erythrocytes in the blood stream. It is familial and congenital and is found most frequently, but not exclusively, in southern European races, the Greek,

Syrian, Italian, and Armenian peoples being most commonly affected. The racial incidence is so pronounced that the disease has been called Mediterranean anemia or *Thalassemia*. Recent reports reveal that it occurs more frequently in other races than was first supposed. Because of the small thin erythrocytosis it has also been termed *Familial Leptocytosis*.

The pathogenesis is not well established. The disease has been considered as (1) an anemia which is due primarily to an increased destruction of erythrocytes with compensatory hyperplasia of the bone marrow, (2) a primary hematopoietic defect with the production and liberation of immature erythrocytes and a compensatory hemolytic process, and (3) a primary defect in pigment metabolism. Because of the similarity of many of its features to those of other types of hemolytic anemia in children the disease has been temporarily placed in this category until more definite information about its pathogenesis has been discovered.

Pathology

There is a marked hyperplasia of the cellular elements of the bone marrow with erythropoietic hyperplasia predominating. Large phagocytic cells having a foamy cytoplasm have been found in the bone marrow in some cases. The spleen is large and fibrotic with the follicles compressed by the increased pulp and interstitial tissue. Infarcts are frequently encountered. There may be foci of extramedullary hematopoiesis involving erythrocytes, myelocytes, and megakaryocytes. Pigment deposits are present in the spleen and liver as well as in all other parenchymatous organs.

Clinical Picture

The disease in its more severe form appears in infancy or childhood and may be acute or chronic in its course. The symptoms are those of the anemia with weakness, fatigability, and general ill health associated with a poor nutritional and developmental state. There is pallor with varying degrees of jaundice and frequently a puffiness of the eyelids. The cranial bones are thickened and the head appears to be unusually large. The cheek bones are prominent and this, together with the above features, produces the "Mongolian" facial appearance. The thickening of the skull and cheek bones is due to the hyperplasia of the marrow occurring at an early age. The spleen is enlarged and firm and the liver becomes enlarged in the later stages of the disease. The enlargement of these organs causes a prominence of the abdomen which is made more apparent by the undernourished state of the child. Ascites and pleural effusion may develop.

Roentgenologic examination of the bones shows the medullary trabeculations to be unusually prominent, especially in the ribs, metacarpals, and metatarsals. The cortex becomes thin with occasional punched-out areas due to invasion of the cortex by the hyperplastic marrow substance. There is a thickening of the medullary portion of the skull with a thinning of the outer and inner tables which give a spongy or mottled appearance (Fig. 40). Fine perpendicular striations are apparent when the skull is viewed in profile. The bones of the face are thickened. The pelvic and other flat bones show rarefaction and irregular trabeculations (Fig. 41). The long bones are thicker than normal because of the increase in the medullary portion, but the cortex is very thin. There is no involvement of the joints and no periosteal elevation. These findings are common in erythroblastic anemia but are not specific for it. Similar changes are found in other forms of hemolytic anemia in children.

The condition occurs in a mild form which is frequently asymptomatic and which is encountered in adults as well as in children. Many of these mild forms have been discovered in testing the blood of parents or siblings of children with obvious manifestations of the disease and finding the characteristic hematologic changes in subjects who have few if any symptoms referable to this disease. We have encountered these mild forms among the families of students whose anemia was discovered during routine student health examinations and who were entirely unaware of the disease. This mild form of anemia occurs with much greater frequency than was formerly supposed.

Hematologic Features

The blood picture is characterized by a moderate or severe grade of anemia with a color index much below normal. There is an associated leukocytosis with counts ranging from 20,000 to as high as 50,000. The platelets are normal. The most striking feature—the presence of great numbers of nucleated erythrocytes in the blood stream—has resulted in the term *erythroblastic anemia* being applied to the disease. In addition to the nucleated erythrocytes there is a moderate degree of polychromatophilia and a reticulocytosis. Marked variations in the size and shape of the erythrocytes occur and achromia is a striking feature. The predominant cell is small and irregular and the anemia is definitely microcytic in type. Target cells are frequently encountered. These are erythrocytes with a dark central area, a light staining intermediate ring, and a dark peripheral zone. The fragility of the red cells to hypotonic salt solution is decreased. Immature leukocytes of the myeloid series appear so that myelocytes and metamyelocytes are often found in addition to an in-

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creased percentage of nonsegmented neutrophils. Increased bilirubinemia is shown by the van den Bergh reaction, and the icterus index is elevated. The urobilinogen content of the feces is increased.

In the mild form of the disease, *Thalassemia minor*, clinical manifestations are slight or absent and many cases are first recognized when hematologic studies are done on other members of a patient's family or on patients with a mild unexplained anemia. In this mild form of the disease one of the most striking hematologic features is the very low color index with erythrocytes which are small, pale and many of which are irregularly shaped. The variation in the size of the erythrocytes is a striking feature but target cells are not prominent. In addition to the low color index and the low mean corpuscular hemoglobin there is also a reduction in the volume index and mean corpuscular volume. The erythrocyte count may be normal or but slightly lowered whereas the hemoglobin level is considerably below normal but it does not increase with iron therapy. The fragility of the erythrocytes to hypotonic saline solution may be decreased. There are all gradations in the hematologic picture from one which is practically normal to that which has been described for the severe form of Cooley's anemia. The disease must be borne in mind when one is confronted by a patient with an unexplained hypochromic anemia.

Prognosis

The course of this disease in children is progressive, without remissions, and death usually occurs before puberty. The patients frequently die of an intercurrent infection since their resistance is very low. The course may be rapid, particularly when the disease appears during infancy. As is true in congenital hemolytic icterus the less severe forms of the disease do not become manifest until adolescence or adult life. They may be nearly or entirely asymptomatic and may be discovered only in a survey of the family of a known case of Cooley's anemia. The anemia may be mild with but slight reduction of the erythrocyte count although the hemoglobin and hematocrit are more markedly reduced. The hypochromia, the microcytosis, and the variations in the shape of the erythrocytes are the outstanding features. When the condition is found in adults the course is extremely chronic, frequently asymptomatic, and may cause no disability whatsoever.

Treatment

No type of therapy has been found to be successful in this disease. Transfusions are palliative, giving only temporary relief, and the usual medications for anemia are without benefit. Splenectomy has been tried but is not fol-



FIG 40 Roentgenogram of the skull of a child with erythroblastic anemia showing mottling and thickening. (Cooley, Witwer, and Lee, *Am. J. Dis. Child.*)

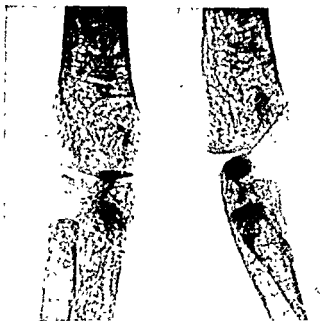


FIG 41 Roentgenogram of the bones of a child with erythroblastic anemia showing a diffuse loss of density and increased trabeculations. (Cooley, Witwer, and Lee, *Am. J. Dis. Child.*)

the Rh factor or agglutininogen. This Rh factor is found in the blood of the Rh+ father. The Rh factor is inherited as a dominant mendelian character. In the Rh- mother erythroblastosis it acts as a sensitizing agent. The Rh- mother the fetus may have Rh+ blood. The mother (Rh-) would become sensitized to the Rh+ erythrocytes of the fetus and develop anti-Rh agglutinins. These penetrate the placenta and hemolyze the erythrocytes of the fetus. The hematopoietic system of the fetus becomes hyperplastic in an attempt to produce enough cells to compensate for those being hemolyzed.

The explanation of erythroblastosis is not as simple as the early explanation, given above, would indicate since there have subsequently been discovered eight combinations of Rh agglutinogens in the cells and also an Hr antigen which is capable of causing isosensitization. It has also been shown that the A and B agglutinogens may be antigenic and cause isosensitivity. Not all infants with an Rh+ father and Rh- mother have erythroblastosis since about half of the Rh+ men are heterozygous, having the dominant Rh gene together with the recessive rh (Rh rh). Only half of their offspring would be Rh+ and only half of these would develop anti-Rh agglutinins.

It is also possible for the mother to become sensitized to the A or B agglutinogens and this type of isosensitivity accounts for a few cases of erythroblastosis. A clinical example of this is given in the case of a child born to an Rh positive mother. It is also possible for the mother to become sensitized to the A or B agglutinogens and this type of isosensitivity accounts for a few cases of erythroblastosis.

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McGowan in Chapter XXIII discusses the development of such immune bodies will have hemolytic transfusion reactions if given Rh+ blood. The donor's blood must be tested for the presence of the Rh factor whenever the mother of a child with erythroblastosis is to be given a transfusion. This theory of the development of erythroblastosis explains many features of the disease.

Search for the Rh factor in the blood of parents of erythroblastic fetuses has shown the necessary combination of Rh+ father and Rh- mother in a very high percentage of the cases.

Further studies are being made to determine the exact mechanism of the disease.

lowed by any lasting improvement. It results only in a rapid and prolonged increase in the number of nucleated erythrocytes in the blood stream.

ERYTHROBLASTOSIS FETALIS

Erythroblastosis fetalis or hemolytic disease of the newborn is a condition resulting from an excessive destruction of erythrocytes. The hemolysis occurs in varying degrees of severity, beginning in utero and becoming manifest at the time of birth or soon thereafter. In the severe forms it results in a stillbirth and miscarriage whereas in less severe cases the child is born with varying degrees of jaundice and anemia. The clinical classification has separated the disease into the three categories of hydrops fetalis, icterus gravis neonatorum, and congenital anemia depending upon the severity of the disorder but all are included in the term erythroblastosis fetalis. The manifestations are those of concomitant erythrocytic destruction and regeneration so that jaundice and anemia are predominating features. Because of the rapid regeneration of blood there are extreme degrees of extramedullary hematopoiesis and in the peripheral blood there are many nucleated erythrocytes, reticulocytes, and polychromatophilia. The great number of nucleated erythrocytes is the most striking feature.

The pathogenesis of this condition has been definitely established within the past few years as being an antigen-antibody reaction in which isosensitization is produced in the mother by the development of antibodies against the erythrocytes of the fetus. The isosensitization develops in the mother when cells of the fetus pass the placental barriers and gain access to the maternal circulation or, in some cases, the mother has previously been sensitized by means of blood transfusions. When the mother's serum containing the agglutinins diffuses through the placenta and comes in contact with the erythrocytes of the fetus they are hemolyzed.

The isosensitization depends in most instances upon the Rh system of agglutinogens in the erythrocytes which is entirely separate from the ABO system by which the usual blood types are determined. When the blood of a rhesus monkey is injected into a guinea pig, an antibody is developed in the guinea pig's serum which will agglutinate the erythrocytes of the monkey. This antiserum from the guinea pig will also cause agglutination of the erythrocytes in about 85 per cent of humans. Such persons are Rh positive (Rh+), and their erythrocytes contain the Rh factor or agglutinin. In the remaining 15 per cent of the population there is no agglutination with the antiserum, they are Rh negative (Rh-), and their erythrocytes do not contain

of erythroblastosis is established during pregnancy there is nothing that can be done to prevent it or to lessen its severity although provisions can be made to have Rh— blood available for transfusions if needed.

Hydrops Fetalis

Hydrops fetalis—universal edema of the fetus or congenital hydrops—is the most severe of the erythroblastoses. Its incidence is difficult to determine, but in a large series of obstetrical cases Hellman and Hertig found it occurring once in 1200 to 2000 deliveries. Javert, considering all dead fetuses above 1500 Gm in weight and all infants dying in the first two weeks of life, found that it accounted for 4.5 per cent of all fetal and infant deaths. The diagnosis is probably missed in many cases, particularly in premature still-born infants which appear essentially normal. Dystocia is common since the edema increases the size of the fetus, and it is not unlikely that some stillbirths which are apparently due to difficult labor are actually examples of this condition. The edema may manifest itself on roentgenographic examination by massive soft tissue shadows and by a corona about the skull due to the edematous scalp. At the time of birth a yellow amniotic fluid and yellow vernix caseosa may call attention to the possibility of erythroblastosis, but these findings are not diagnostic of the condition. The mortality of hydrops fetalis is 100 per cent, the fetus usually being stillborn or making only a few feeble attempts at respiration. The infant presents a waxy skin, a marked pallor, and multiple areas of subcutaneous hemorrhage. The edema is usually extensive so that even a premature infant is large. The abdomen is enlarged and swollen both from the ascites and from the enlarged liver and spleen. Jaundice is seldom present but may appear in a very mild degree. Complete blood studies are seldom obtained because of the frequency of stillbirths, but the findings are similar to those of icterus gravis neonatorum.

Pathologic examination reveals not only the subcutaneous edema but collections of fluid in all serous cavities. Petechiae are common on the serous surfaces and in the internal organs. The liver is enlarged and shows necrosis, deposits of iron-containing pigment, and many scattered islands of hematopoiesis which are both erythroblastic and myeloblastic. The spleen is likewise enlarged and contains areas of erythrocytic and myelocytic activity, the former predominating. Similar evidences of hematopoiesis may be found in almost all of the organs. Slight extramedullary hematopoiesis is frequently present in a newborn infant but in erythroblastosis it far exceeds the normal. The bone marrow is markedly hyperplastic. The placenta is large and edematous, and the vessels contain many immature erythrocytes.

lytic anemia: (1) anemia, which varies in its severity, resulting from the rapid destruction of erythrocytes but which is modified to some extent by the rapidity of erythrocytic regeneration; (2) jaundice which is due to liberation of pigments from the hemolyzed erythrocytes; (3) evidences of regeneration of the erythrocytes as shown by a high reticulocyte count, polychromatophilia, and nucleated erythrocytes; and (4) leukocytosis with the appearance of immature cells of the myeloid series in the blood stream.

The three forms of erythroblastosis fetalis present identical pathologic changes although their clinical features and prognosis vary. Hydrops fetalis or universal edema of the fetus is the most severe form and results in a premature stillbirth or death within a few hours after birth. Icterus gravis neonatorum presents jaundice which is present at the time of birth or appears very soon thereafter. This too may result in a stillborn infant, but more frequently death occurs during the first two weeks of extrauterine life. Congenital anemia, the third and least severe form of the erythroblastoses, presents a severe anemia, but jaundice and edema are usually absent. The clinical and hematologic features of the three entities may overlap so that there are some cases which do not fall clearly into any one of the specific types.

Erythroblastosis fetalis does not appear in a primiparous mother except in those instances in which isosensitivity has been produced by preceding blood transfusions. A single transfusion may produce permanent sensitivity to the Rh agglutinin. The first offspring is usually normal in the absence of a preceding transfusion, since the mother has not yet developed agglutinins in high enough titer to produce hemolysis but following one or more normal children there occur premature infants, stillbirths, or infants with erythroblastosis. Succeeding pregnancies usually result in progressively more severe forms of the disease but this is not always true. Normal infants may sometimes follow erythroblastotic children. With a history of repeated stillbirths, miscarriages, or premature births in a nonsyphilitic mother one must suspect the possibility of erythroblastosis. When erythroblastosis has made its appearance, there is about an 80 per cent probability that subsequent pregnancies will have a similar termination. Should the mother require transfusions at any time it must be from an Rh— donor for otherwise a severe hemolytic reaction may ensue.

Erythroblastosis can be predicted before delivery in most but not all instances when Rh typing by the newer methods is done. It is impractical to type all pregnant women for the Rh factor but it should be done in those who have received transfusions and in those who have previously had a stillbirth or delivered a premature or jaundiced infant. Even though the probability

birth, but jaundice and edema are mild or absent. The liver and spleen are large but not so massive as in congenital hydrops or icterus gravis neonatorum. Examination of the blood reveals an anemia of varying degree, and the smear gives a picture similar to that of icterus gravis neonatorum but with fewer nucleated erythrocytes and a leukocytosis which is not so high. There are fewer immature cells to be found. Pathologically the picture is the same as previously described, but the changes are less extensive. Only mild extramedullary hematopoiesis and hyperplasia of the bone marrow are encountered. The prognosis for prompt and complete recovery is good.

There is a striking similarity in the pathologic picture and the clinical aspects of the erythroblastoses, of Cooley's erythroblastic anemia, and of familial hemolytic icterus when it appears in a severe form in infancy so that differentiation of these conditions may be difficult. The racial tendency and the age of the patient aid in distinguishing Cooley's anemia. Increased fragility of the erythrocytes together with the family history in hemolytic icterus are characteristic. Extramedullary hematopoiesis, which is so common in erythroblastosis fetalis, may be found to a limited extent in the other conditions.

Treatment

The treatment consists in the administration of Rh- blood in sufficient amounts to maintain a normal hemoglobin and erythrocyte level. The mother's blood, which contains the anti-Rh factor, cannot be used to transfuse the infant although the mother's washed erythrocytes may be given. Excessive amounts of blood are not of benefit and "replacement transfusions" are not advisable. The frequency with which transfusions are given and the duration of the treatment depend upon the severity of the anemia. In most instances there is a marked improvement after the first week. When recovery occurs, as it does in about 65 per cent of the cases, it is permanent and complete.

A few cases have been reported in which cirrhosis of the liver and Banti's syndrome have developed in children who presumably had had erythroblastosis and it is possible that this condition may lead to permanent liver damage in some cases. Kernicterus, a degenerative change in the brain, may result and the question has been raised as to whether this is a result of the severe icterus and pigment deposits in the cells or whether it results from multiple small thrombi as a result of agglutinated erythrocytes.

VON JAKSCH'S ANEMIA

Von Jaksch's anemia or anemia pseudoleukemia . . .
not a distinct disease entity but a symptom complex ap.

Icterus Gravis Neonatorum

Icterus gravis neonatorum presents a pathologic picture similar to that of hydrops fetalis but differs in its clinical aspects. Stillbirths are not as frequent as with hydrops fetalis. Jaundice, the most outstanding clinical feature of this condition, is present at birth or appears very soon thereafter and becomes progressively deeper. It is more severe, more progressive, and of longer duration than the physiologic icterus of the newborn, which does not have the associated hematologic or clinical features. Although edema is present in many cases of icterus gravis neonatorum, it is not constant. The liver and spleen are markedly enlarged.

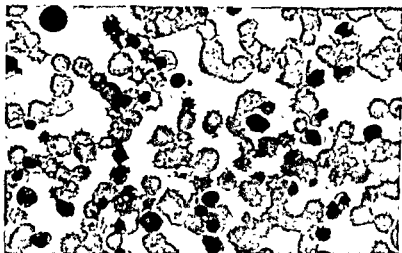


FIG. 42. Photomicrograph of the blood from a patient with icterus gravis neonatorum showing the great number of nucleated erythrocytes.

There is a varying degree of anemia—usually quite severe—with a normal or high color index. The leukocyte count is elevated. The smear shows enormous numbers of nucleated erythrocytes in all stages of development (Fig. 42). Polychromatophilic erythrocytes and reticulocytes are numerous. *The elevated leukocyte count is due to an increase in the number of granulocytes, some of which may be immature.*

Necropsy reveals a pathologic picture similar to that of hydrops fetalis except that edema is lacking and the extramedullary hematopoiesis is less extensive.

Congenital Anemia

Congenital anemia is the least severe of the three forms of erythroblastoses and carries a far better prognosis. Pallor and a severe anemia are present at

birth, but jaundice and edema are mild or absent. The liver and spleen are large but not so massive as in congenital hydrops or icterus gravis neonatorum. Examination of the blood reveals an anemia of varying degree, and the smear gives a picture similar to that of icterus gravis neonatorum but with fewer nucleated erythrocytes and a leukocytosis which is not so high. There are fewer immature cells to be found. Pathologically the picture is the same as previously described, but the changes are less extensive. Only mild extramedullary hematopoiesis and hyperplasia of the bone marrow are encountered. The prognosis for prompt and complete recovery is good.

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Treatment

The treatment consists in the administration of Rh- blood in sufficient amounts to maintain a normal hemoglobin and erythrocyte level. The mother's blood, which contains the anti-Rh factor, cannot be used to transfuse the infant although the mother's washed erythrocytes may be given. Excessive amounts of blood are not of benefit and "replacement transfusions" are not advisable. The frequency with which transfusions are given and the duration of the treatment depend upon the severity of the anemia. In most instances there is a marked improvement after the first week. When recovery occurs, as it does in about 65 per cent of the cases, it is permanent and complete.

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VON JAKSCH'S ANEMIA

Von Jaksch's anemia or anemia pseudoleukemia infantum is probably not a distinct disease entity but a symptom complex appearing in infancy

as an abnormal response of the hematopoietic system to any one of various types of stimuli. Von Jaksch's original description was not clear but it stressed an anemia of the hypochromic type in infancy, variations in the size and shape of the erythrocytes, enlargement of the spleen and liver, and extreme leukocytosis. The term has been used rather loosely and applied to almost any type of severe anemia in infants which was not readily explained. The syndrome has been found in association with syphilis, tuberculosis, and other infections, in celiac disease, rickets, and dietary deficiencies as well as in association with hemolytic anemias. It is now realized that the hematopoietic system of an infant is more labile than that of an adult so that the response to any severe anemia may be characterized by the appearance of nucleated erythrocytes and a leukocytosis with immature cells.

There are many who would drop the term *von Jaksch's anemia* entirely, others believe it to be a specific disease entity or at least a specific congenital defect of hematopoiesis which produces this peculiar response to a variety of stimuli. The most prevalent view is that it is an exaggerated infantile response to a severe anemia or infection.

OVALOCYTOSIS

Ovalocytosis is a rare hereditary defect of hematopoiesis characterized by the presence of large numbers of oval or elliptical erythrocytes in the blood stream. It is equally common in males and females, may be transmitted by either sex, and has been found in both white and Negro patients at all ages.

Although ovalocytosis is usually asymptomatic, in some cases it is associated with a moderate or severe grade of anemia which is unexplained except by the abnormality of the erythrocytes. Definite conclusions as to the exact significance of ovalocytosis cannot be drawn from the relatively few reported cases, but the evidence suggests that in many respects it is analogous to sickle cell anemia. In either condition the defect in the erythrocytes may be present with no anemia and no clinical evidence of disease although sometimes it is accompanied by anemia.

The erythrocyte count and hemoglobin value vary, but examination of the blood smear reveals the characteristic alterations in the shape of the cells. A majority of the erythrocytes are oval, elliptic, or sausage shaped, a few assume more bizarre forms (Fig. 43). The reticulocytes are not increased in number, and the resistance of the cells to hypotonic saline is not changed.

TARGET CELL ANEMIA

Target cells are erythrocytes with a peripheral rim of hemoglobin-containing cytoplasm, a central hemoglobin-containing area, and between these an unstained ring which contains little hemoglobin (Fig. 44). They are occasionally encountered in chronic anemia of any type and are numerous in most cases of Cooley's erythroblastic anemia and sickle cell anemia. They are common in the early stages of blood regeneration.



FIG. 43 Photomicrograph of a blood smear showing ovalocytosis (Hal Downey Handbook of Hematology, Paul B. Hoeber, Inc.)

Target cell anemia is the name that has been applied to a condition which undoubtedly represents a mild form of Cooley's anemia or *Thalassemia minor*.

HEMOGLOBINURIA

Hemoglobinuria is a result of rapid intravascular destruction of erythrocytes. Under normal conditions these cells are being continuously removed

from the circulation and destroyed by the reticulo-endothelial tissues. The released hemoglobin is catabolized through certain ill-defined stages into bilirubin which is excreted by the liver. Only a very small amount of free hemoglobin is found in the plasma under these conditions wherein the erythrocytes are destroyed intracellularly by reticulo-endothelial cells. Even though this destruction of erythrocytes is markedly accelerated, as in hemolytic anemias, the excess hemoglobin is broken down and there results only an increased concentration of plasma bilirubin and an increased excretion of urobilinogen

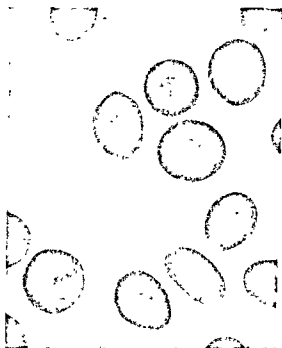


FIG. 44 Target cells

There is only a moderate elevation in the free hemoglobin content of the plasma as a rule.

In certain hemolytic processes the destruction of the erythrocytes apparently takes place outside the reticulo-endothelial cells, and is a true intravascular hemolysis with hemoglobin being liberated directly into the blood plasma. When this hemolytic process is rapid the hemoglobinemia reaches concentrations above the threshold level, which apparently is about 175 mgm. per 100 cc., and hemoglobinuria results. The hemoglobin undergoes chemical changes upon its liberation and a part is converted into methemoglobin, hematin, and possibly other pigment derivatives which also appear in the

urine. Some of the hematin which is liberated into the blood stream combines with the plasma albumin to form methemalbumin, which is more toxic than other hemoglobin derivatives. Methemalbumin appears in the urine only under abnormal conditions when there is an extensive hemolysis of erythrocytes and a high hemoglobin content of the blood plasma. In hemoglobinuria, therefore, there is not only hemoglobin in the urine but other pigment derivatives of hemoglobin which impart the dark reddish brown color. Albuminuria with hyaline, granular, and pigment casts are also found. Since hemosiderin is present in the urine the addition of a small amount of dilute ammonium sulfide to the urine sediment will cause the precipitation of black granules of ferrous sulfide which are identifiable on microscopic examination.

Rapid intravascular hemolysis of erythrocytes and hemoglobinuria is frequently accompanied by severe renal damage with resultant anuria, uremia, and death. Experimental observations in animals following the injection of solutions of hemolyzed erythrocytes have shown this same sequence of events if the urine was acid in reaction. Histologic examination of these animals' kidneys revealed many renal tubules occluded with hemoglobin casts. If the urine was alkaline in its reaction there were no ill effects in the animals and the kidneys were normal in their appearance. Other experiments have indicated that pure hemoglobin is harmless to the kidney but that with an acid reaction of the urine methemoglobinuria produced a marked reduction in clearance values and tubular necrosis. These results suggest a primary damage to the tubular epithelium rather than plugging of the lumen of the tubules. Spasm of the renal vessels has also been noted with a reduction in the number of functioning glomeruli. Regardless of the mechanism by which it is produced, the fact remains that hemoglobinuria all too frequently causes severe renal damage and uremia.

A paroxysm of hemoglobinuria, if severe, is usually accompanied by aching pains in the back and legs, abdominal cramps, headache, malaise, and prostration. Fever, accompanied by a chill, appears soon after the onset of hemolysis, usually before the dark urine is detected by the patient. There may be increased hemoglobinemia without hemoglobinuria if the amount of hemolysis is slight. The hemoglobin in the blood is soon changed to bilirubin so that jaundice will become evident if this accumulates above the excretory capacity of the liver. The degree of jaundice does not necessarily parallel the degree of anemia or the amount of hemolysis. The jaundice is of the acholuric type.

Vascular disturbances, similar to those found in Raynaud's disease, frequently accompany hemoglobinuria and are probably due to thrombotic oc-

clusion of peripheral vessels. Thromboses are frequently encountered in the vessels of the spleen, the retinal and mesenteric vessels, and in those of the extremities. Such thromboses must be considered, with shock and anuria, as one of the grave dangers of any hemolytic process.

Anemia develops rapidly with the destruction of the erythrocytes. Its severity depends upon the number of cells hemolyzed. When the episodes of hemolysis subside, a rapid regeneration of cells and hemoglobin takes place *since the function of the bone marrow has not been altered and there is abundant material for the formation of new erythrocytes*.

Toxic Hemoglobinuria

Rapid hemolysis of erythrocytes to such extent that hemoglobinuria results may occur with a number of parasitic infestations such as malaria (black water fever) or *Bartonella* (oryza fever). It also results from certain chemical agents and drugs. One of the most common of these is the sulfonamide group of drugs, of which all have been incriminated, with sulfanilamide the most frequent offender. Among others are phenylhydrazine, arseniureted hydrogen, dinitrobenzol, aniline, saponin, trinitrotoluene methyl chloride, benzene, promin, and quinine. Arsine, a war gas, is rapidly absorbed from the respiratory tract and almost immediately damages the erythrocytes so that they are rapidly hemolyzed. This gas may be accidentally generated when an acid and metal, either of which may contain arsenic, are brought in contact and hydrogen is liberated. It is therefore a hazard in certain industries.

Rapid hemolysis of erythrocytes and hemoglobinuria follow the bites of *certain snakes and spiders*.

Hemoglobinuria of Burns

Hemoglobinuria frequently occurs in patients with severe burns and represents a bad prognostic sign. The erythrocytes passing through the heated area at the time the burn occurs are damaged and subsequently are destroyed with a resultant hemoglobinuria and hemoglobinemia. Experimental work has shown that heating blood to a temperature above 51° produces irreversible changes in the erythrocytes which result in alterations in their fragility, and that *transfusion of heated blood into normal animals results in hemoglobinuria*. Fragmentation of erythrocytes and alteration in the osmotic fragility of the cells have been observed in severely burned patients.

March Hemoglobinuria

Paroxysmal hemoglobinuria as a result of exertion is a rare condition which has been observed most frequently among soldiers. Young adult males appear

to be most frequently affected. As a rule they show no other evidence of disease. It has been noted that the attacks occur after exercise in the upright position, especially walking or marching, and cannot be reproduced by other types of equally strenuous exertion in other positions. Exposure to cold does not initiate an attack. In many respects this disease resembles orthostatic albuminuria, and lordosis has been considered as a possible etiologic factor. Increased lactic acid production with a resultant alteration of the pH of the blood does not explain the hemolysis since all types of exercise do not produce the reaction.

There are no systemic manifestations as a rule although a dull ache may appear in the lower back or abdomen. The urine becomes red or brown soon after the exercise and the discoloration persists for several hours, but has been known to last for several days. The free hemoglobin of the plasma is increased but the hemolysis is not extensive enough to produce anemia or jaundice.

This condition may represent an accentuation of a physiologic hemoglobinemia and hemoglobinuria which occurs in healthy individuals following prolonged exertion. An increase in plasma hemoglobin has been demonstrated in athletes after long distance races, and a few have shown hemoglobinuria. Those patients having march hemoglobinuria probably develop this hemoglobinemia with less strenuous exercise than do other individuals and this may be due to a lowered renal threshold for hemoglobin. This explanation, offered by Gilligan and Blumgart, does not explain the influence of body posture and position in the production of hemoglobinuria.

There is no effective treatment for the condition. The disease tends to disappear spontaneously within a period of a few months or a year, the paroxysms becoming less frequent and less severe and finally disappearing entirely.

Paroxysmal Nocturnal Hemoglobinuria

Paroxysmal nocturnal hemoglobinuria or Marchiafava-Micheli syndrome is a rare condition in which hemolysis of erythrocytes and the consequent hemoglobinuria occur during sleep. It appears most frequently in males in the third and fourth decades of life. There is no family history of a similar disease and no association with infections such as syphilis or malaria.

Much speculation has been aroused as to the cause of the hemolysis. The work of Ham and his collaborators has postulated the pathogenesis to be the result of changes in the acidity of the blood during sleep. Patients with this disease were kept awake for long periods of time, and no hemoglobinuria appeared even when they were resting under basal conditions. The typical

anifestations appeared, however, when the patient slept, regardless of whether this was during the day or night. During sleep there is stated to be slight decrease in the alkalinity of the blood as a result of an increase in its carbon dioxide content. Recent workers, however, have shown that the acidity of the blood seldom falls below a pH of 7.2 during sleep and in some cases of paroxysmal nocturnal hemoglobinuria there has been no detectable fall in the hydrogen-ion concentration. There is no experimental proof that the action of the blood in the viscera falls to a level low enough to cause hemolysis. The administration of an acid salt—ammonium chloride—increased the hemoglobinuria. The hemoglobinuria decreased when an alkaline salt—sodium bicarbonate—was given but became worse when the alkali was withdrawn. The fundamental defect appears to be in the erythrocytes. They are usually susceptible to hemolysis in serum of increased acidity even though the acidity is still within the normal physiologic range. Cells from normal individuals are not hemolyzed by acidified serum or by the patient's serum, but acidified serum from a normal person hemolyzes the patient's cells. Although the pathogenesis of this condition is still unsettled there has been no better hypothesis advanced than that of Ham's.

Initial Manifestations

The disease usually has an insidious onset with increasing pallor and varying degrees of jaundice although pains in the back, legs, or abdomen may be the initial manifestation. The passage of dark-colored urine may be the first evidence of disease to be noticed by the patient, or the hemoglobinuria may appear only after other symptoms have been present for some time. The disease is characterized by remissions and exacerbations. There is an increasing degree of anemia and the appearance of jaundice during the periods when paroxysms of hemoglobinuria are of frequent occurrence. Each paroxysm of hemolysis and hemoglobinuria is characterized by chills and fever, and pain in the abdomen and in the lumbar region. The patient becomes temporarily disabled. The attack may last for only one night, or there may be repeated episodes for a period of weeks.

The hemoglobinuria is paroxysmal in its occurrence but hemosiderin is apparently excreted at all times by these patients, and is more typical of the disease than is hemoglobinuria.

Venous thromboses, frequently in the portal system, occur in about 25 per cent of the patients and present one of the most serious complications of the disease. These usually occur during the acute exacerbations, but have also

been encountered during a remission. The cause of the thromboses has not been adequately explained although it has been suggested that accumulations of erythrocyte stroma may lead to the occlusion.

The anemia may be mild, but if the attacks are of long duration it becomes more severe. There is a reduction in the number of leukocytes and platelets during the hemolytic episode, but the reticulocytes are more numerous and polychromatophilia is prominent. Nucleated erythrocytes are frequently encountered. The fragility of the erythrocytes to hypotonic saline is normal. The urine becomes red or very dark brown during the paroxysm. Hemoglobin, methemoglobin, and hematin are present. There is an elevated hemoglobin content of the blood plasma and soon a hyperbilirubinemia develops with increased urobilinogen in the feces.

The course of the disease is long and protracted, one case having been followed for thirty-three years. Death usually results from other diseases.

Pathology

The pathologic changes are those associated with increased hemolysis of the red cells. The spleen is enlarged but is not congested as in hemolytic icterus. There are thromboses of the central veins of the liver with zonal hepatic necrosis. Large amounts of iron-containing pigment are deposited in the tubular epithelium of the kidneys. The bone marrow shows hyperplasia with a predominance of normoblasts.

Diagnosis

The diagnosis is based on the occurrence of hemoglobinuria during sleep and the inability to produce hemoglobinuria by exposure to cold or by exercise. The hemolysis test in which the patient's cells are subjected to acidified serum is of help in establishing the diagnosis as is the demonstration of the persistent presence of hemosiderin in the urine. The normal fragility of the erythrocytes when exposed to hypotonic saline is of help in excluding hemolytic icterus—a disease with which it is frequently confused. A careful check for nocturnal hemoglobinuria should be made in all cases of atypical hemolytic anemia.

Treatment

There is no adequate treatment available. Alkaline salts may diminish the hemoglobinuria and be of some temporary value, but the end results are not satisfactory, the symptoms recurring when the alkali is stopped. Splenec-

manifestations appeared, however, when the patient slept, regardless of whether this was during the day or night. During sleep there is stated to be a slight decrease in the alkalinity of the blood as a result of an increase in its carbon dioxide content. Recent workers, however, have shown that the acidity of the blood seldom falls below a pH of 7.2 during sleep and in some cases of paroxysmal nocturnal hemoglobinuria there has been no detectable fall in the hydrogen-ion concentration. There is no experimental proof that the reaction of the blood in the viscera falls to a level low enough to cause hemolysis. The administration of an acid salt—ammonium chloride—increased the hemoglobinuria. The hemoglobinuria decreased when an alkaline salt—sodium bicarbonate—was given but became worse when the alkali was withdrawn. The fundamental defect appears to be in the erythrocytes. They are unusually susceptible to hemolysis in serum of increased acidity even though this acidity is still within the normal physiologic range. Cells from normal individuals are not hemolyzed by acidified serum or by the patient's serum, but acidified serum from a normal person hemolyzes the patient's cells.

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Pathogenesis

Syphilis, of either the congenital or the acquired type, is now accepted as the primary cause of this disease. It has been found in children with congenital syphilis but is more common in adults with a late stage of the acquired form. The Wassermann reaction is almost invariably positive, but many patients show no other evidences of syphilis. The hemoglobinuria is caused by the rapid hemolysis of great numbers of erythrocytes in the blood stream so that hemoglobin accumulates in the plasma to a point above the renal threshold. It is then excreted in the urine. The hemolysis is brought about by the presence of an autohemolysin which is capable of hemolyzing the red blood cells under certain conditions.

The Donath-Landsteiner reaction demonstrates the fundamental mechanism of this hemolytic reaction. It is the test on which the diagnosis of this disease must be based. To a suspension of the patient's cells in normal saline is added some of the patient's own serum. Half of the preparation is placed in an incubator at 37 C. The other half is chilled to 5 C.—placed in ice water—for twenty minutes and then put in the incubator. Hemolysis occurs in the specimen which was chilled but does not occur in the other. The reaction is not present in normal blood. This autohemolysin is of the "immune body" type and depends upon an amboceptor, which combines with the red cells only at a low temperature, and a complement, which can act on the cells only after the amboceptor has combined with them. After the blood has been chilled, the complement in the serum causes hemolysis when the temperature is raised.

The blood in the superficial skin vessels becomes chilled when the patient is exposed to low temperatures. Hemolysis results when this chilled blood passes to the higher temperatures of the internal vessels. The paroxysm of hemolysis can be artificially produced by exposing the patient to cold (16–18 C.) or by immersing the forearm or foot in ice water. In some instances the hemolysis is slight, and although free hemoglobin appears in the blood plasma, it does not pass into the urine.

Symptoms

Following exposure to cold there is a latent period before the symptoms appear. This varies from a few minutes to several hours. The symptoms consist of chills, fever, malaise, headache, and severe pains in the legs, lower back, and occasionally the abdomen. The episode lasts for a few hours and may be accompanied by cyanosis. The temperature may rise to 104 F. or

tomy has been performed but does not alter the course of the disease. It should not be advised.

Favism

Favism is a disease characterized by acute episodes of hemoglobinuria which, in susceptible individuals, follow exposure to certain beans (*Vicia fave*). The exposure may consist of inhalation of the pollen from the blossom of the bean plant or ingestion of the beans, either cooked or raw. The disease is rare in this country but is prevalent in Sicily and southern Italy. The hypersensitiveness to the plant is hereditary. Most patients give a family history of the disease.

Favism may appear in children or in adults. Some persons may eat the beans for years without trouble and then suddenly develop a sensitivity to them. Inhalation of the pollen brought on the attacks in 38 per cent of the reported cases, and 62 per cent resulted from ingestion of the raw or cooked beans.

The symptoms appear from one to six hours after inhalation of the pollen or from one to two days after ingestion of the bean itself. They include fever, weakness, vomiting, muscular twitching, and vertigo. There is a rapid onset of pallor, and jaundice soon becomes evident. The jaundice increases for about the first three days and then subsides. The disease may progress rapidly to unconsciousness and death. Hemoglobinuria appears with the onset of symptoms and may last for three or four days. There may be anuria and uremia in the severe cases. Anemia develops quickly and is at first associated with *leukopenia* and *thrombopenia*.

The course of the disease is usually short. Recovery begins after two to four days, and a majority of the patients improve rapidly. The mortality is about 8 per cent.

Treatment

The treatment is purely symptomatic during the acute phase. Ephedrine is advocated if the patient is in shock. Avoidance of exposure to the bean or the blossoms is the only known method of prevention.

Paroxysmal Hemoglobinuria

Paroxysmal hemoglobinuria is a rare disease characterized by recurring transitory episodes of hemoglobinuria which are brought on in susceptible individuals by exposure to cold.

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over. There may be vasomotor disturbances of the extremities which resemble Raynaud's disease or episodes which are characterized by edema, urticaria, and vesicular lesions. In the severe cases there will be prostration, nausea, vomiting, and abdominal pain. The urine varies in color from red to a very dark brown, described by the patient as being black. This coloring is due to hemoglobin, methemoglobin, and hematin. There is also albuminuria with hyaline, granular, and pigment casts. The hemoglobin may be found in only one urine specimen or it may persist for several days.

Following the acute attack the patient may present a mild grade of jaundice. When the episode results in the destruction of a very large number of erythrocytes, a mild anemia will be present. If the attacks are frequent and severe, the anemia will become more prominent. The patient has no symptoms referable to the disease between the episodes of hemolysis.

Diagnosis

The diagnosis is based on the patient's history of having passed dark urine following exposure to cold, the finding of hemoglobinuria after artificial induction of an attack, a positive Wassermann reaction, and the Donath-Landsteiner reaction. The disease must be differentiated from those hemoglobinurias due to drugs and chemicals, paroxysmal nocturnal hemoglobinuria, and the form which occurs with exertion. None of these are necessarily associated with syphilis, the Donath-Landsteiner reaction is not positive, and immersing an extremity in cold water will not precipitate an attack.

Treatment

There is no specific treatment for the acute attack once it has started except to keep the patient warm and to give what symptomatic treatment is necessary. The patient should be protected against exposure to cold and preferably should change to a warm climate during the winter months. Antisyphilitic treatment should be started immediately and an intensive course of therapy carried out. This will usually result in complete arrest of the symptoms so that the patient can then be exposed to cold without precipitating an attack of hemoglobinuria. The Donath-Landsteiner reaction may remain positive

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BANTI'S SYNDROME CHRONIC CONGESTIVE SPLENOMEGALY

BANTI'S SYNDROME IS A CONDITION CHARACTERIZED BY SPLENOMEGALY, HYPOCHROMIC ANEMIA, LEUKOPENIA, AND ICTERUS WITH ASCITES frequently appearing later in the course of the disease. This symptom complex may be produced by a variety of pathologic states. It is therefore more properly classified as a syndrome than as a disease entity. Exclusion of other diseases which may produce the cardinal features is as essential in the diagnosis of Banti's syndrome as the presence of a specific clinical picture. The condition has been termed Banti's disease, splenic anemia, hypersplenism and chronic congestive splenomegaly.

Etiology

The cause is indefinite in many cases, but the syndrome may result from any condition which causes hypertension in the splenic and portal veins and congestion of the spleen. It has been considered to be an early stage in the development of portal cirrhosis of the liver, but the same clinical picture may occur with other lesions which are either intrahepatic or extrahepatic. Portal cirrhosis of the Laennec type is the most frequent of the intrahepatic lesions which produce Banti's syndrome, accounting for 60 to 70 per cent of all cases.

Increased resistance to the flow of blood through the intrahepatic portion of the portal circulation may be caused by scarring which results from infections or parasitic infestation. Congenital abnormalities of the hepatic vascular system which allow arteriolar pressures to be transmitted directly to the portal circulation may also cause this resistance.

The syndrome is produced by extrahepatic lesions which lead to increased pressure in the splenic vein. This may be due to mechanical blockage from scar tissue, pressure of tumors, congenital abnormalities of the vascular system, or thrombosis of the vein itself as a result of injury or infection. Be-

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slightly reduced in number, but there are usually no hemorrhagic tendencies except those which result from ruptured esophageal varices. In rare instances the platelets are markedly reduced, and the clinical and laboratory features of thrombopenic purpura make their appearance. Hemorrhages may occur from any part of the body when this complication arises.

Hemorrhage from a ruptured varicose vein of the esophagus is frequent and may occur at any time in the course of the disease. Occasionally it is



FIG 45 The esophagus in a patient with Banti's syndrome. The mottled appearance and irregular margins are due to varicosities.

the first recognized symptom of illness. It may be mild, with only slight hematemesis and melena, or it may be severe and rapidly fatal. Roentgenologic examination of the esophagus may reveal an irregular configuration in its lower portion which is indicative of varicosities (Fig 45). These do not interfere with the passage of food and produce no symptoms except when they rupture and bleed.

The size of the spleen will vary from one patient to another. In some

cause of the obstruction to the blood flow there is an attempt to establish a collateral circulation, resulting in varicosities of the esophageal and gastric vessels.

Pathology

The anatomic changes depend upon the causative lesion, but the splenic condition is similar in all cases. It consists primarily of marked congestion with varying degrees of fibrosis. The spleen is enlarged, frequently weighing 800 Gm. or more. It is very firm, the capsule is thickened, and the cut surface has a beefy, fibrotic appearance. Microscopic examination shows dilated sinuses and dense fibrous strands which arise from the periarterial tissues and infiltrate through the reticulum. Siderotic nodules, which result from small hemorrhages, are occasionally found. These are small yellowish brown flecks composed of elastic fibers, intracellular and extracellular deposits of hemosiderin, giant cells, and deposits of calcium. Degenerative changes are sometimes noted in the splenic and portal veins. These vessels are occasionally thrombosed. Congenital abnormalities of the vessels are not infrequent. In one such case a large mass of dilated vessels was found in the hilus of the spleen, and the splenic artery, whose walls were extremely thin, was 2 cm in diameter. An enlarged spleen had been discovered in the patient at the age of 5. When it was removed 28 years later, it weighed 1450 Gm.

The typical anatomic changes of Laennec's cirrhosis are encountered when the primary lesion is a portal cirrhosis of the liver. This disease, with the accompanying fibrosis of the spleen, has been termed hepatolienal fibrosis.

Clinical Features

The symptoms usually make their appearance in early adult life, occasionally in childhood, but only rarely in older adults. In the early stage of the illness there is an enlargement of the spleen associated with anemia and leukopenia. The symptoms are predominantly those of the anemia, namely weakness, fatigability, slight breathlessness, and pallor. The anemia is of the hypochromic type with a low color index. It is relatively mild as a rule with an erythrocyte count of around 3,000,000. It becomes more severe in the late stage or may be intensified at any time as a result of hemorrhage. Anemia is a primary feature of the disease and does not depend upon the loss of blood for its production. The leukopenia is also of moderate degree with a majority of the leukocyte counts ranging between 2500 and 4000. It is due to a reduction in the number of granulocytes so that a differential count shows a relative lymphocytosis without abnormal cells. The platelets are

Since many of the diseases which cause splenomegaly also cause anemia, all such diseases must be borne in mind in the differential diagnosis. Roentgenologic examination of the esophagus may help in establishing a diagnosis if esophageal varices can be demonstrated in a case which is otherwise puzzling. In many instances the diagnosis is speculative rather than firmly established.

Other Causes of Splenomegaly

Many of the causes for enlargement of the spleen are discussed under their respective headings elsewhere in the text and will be merely mentioned at this time. The following represent the most frequently encountered causes for an enlarged spleen and must at least be considered when one is faced with a patient having splenomegaly.

Myelogenous Leukemia of Both Leukemic and Aleukemic Forms

Myelogenous leukemia can be recognized by the changes in the peripheral blood or by examination of material removed from the sternal bone marrow.

Lymphocytic Leukemia in Both Leukemic and Aleukemic Forms

The splenomegaly of lymphocytic leukemia is usually less than in myelogenous leukemia and is associated with a generalized lymphadenopathy. The blood picture or examination of a lymph node removed for biopsy usually reveals the nature of the disease.

Hodgkin's Disease

Hodgkin's disease usually, but not always, causes a lymphadenopathy as well as splenomegaly. The final diagnosis rests on histologic study of a lymph node.

Lymphosarcoma

Immature lymphocytes rarely appear in the blood stream in lymphosarcoma, and the lymphadenopathy is more localized than in either Hodgkin's disease or lymphocytic leukemia. A final diagnosis rests upon histologic examination of a lymph node.

Pernicious Anemia

Splenomegaly occurs in about 50 per cent of pernicious anemia cases, which, because of the leukopenia, icterus, and thrombopenia, are not infrequently confused with Banti's syndrome, particularly when the picture is somewhat atypical.

instances, even in the early stage of the disease, it may be large enough to cause some discomfort such as a heavy dragging sensation in the left upper quadrant of the abdomen. Splenic infarction may occur and cause severe pain over the organ. The spleen increases in size as the disease progresses but usually does not become extremely large. The anemia becomes progressively worse, but the leukopenia does not parallel the erythrocyte count. The liver may be enlarged in the early stage, especially if the disease is caused by portal cirrhosis, but its size diminishes later. The late stage of the disease is characterized by recurring ascites, mild jaundice, and emaciation. The jaundice is not of the obstructive type so that the stools are not acholic and the urine does not contain bile pigments. It is usually of only moderate intensity, giving an indirect van den Bergh reaction. The ascites may be slight or so severe as to require repeated paracenteses. In cases due to primary liver disease, and these constitute a majority, the terminal picture is that of portal cirrhosis with liver insufficiency.

The course of the disease is usually chronic, extending over many years. A fatal termination may occur at any time as a result of gastric or esophageal hemorrhages. The cause of the syndrome determines the course to some extent. In patients in whom it is due to portal cirrhosis the progress is apt to be more rapid than in others. Death, in a majority of these cases, results from failure of hepatic function.

The anemia accompanying Banti's syndrome was formerly considered to be hemolytic in origin because of the associated icterus. However, icterus is not present in the early stage of the disease even though anemia is a prominent feature. The present consensus is that the anemia does not result from increased destruction of erythrocytes, but an adequate explanation for it has not been advanced.

The bone marrow as obtained by sternal puncture presents a variable picture which is not diagnostic of the disease although it serves to exclude certain other causes of splenomegaly. Myeloid hyperplasia has been observed by Limarzi and erythroid hyperplasia and immaturity were also encountered in the late stages of the disease.

Diagnosis

The diagnosis of Banti's syndrome may be difficult since it rests on the exclusion of other diseases associated with splenomegaly, anemia, and leukopenia as well as on the presence of the specific clinical features. Portal cirrhosis and blood dyscrasias such as pernicious anemia and aplastic anemia must be excluded as well as all forms of anemia associated with jaundice.

be enlarged. Splenomegaly occurs in practically all cases. The congo red test may aid in establishing the diagnosis.

Polycythemia Vera

Splenomegaly is present in a majority of patients with polycythemia vera. The spleen may be only slightly enlarged or it may become a very massive organ. The peculiar dusky complexion and the elevated red count and hemoglobin level establish the diagnosis.

Tuberculosis of the Spleen

A rare complication of tuberculosis elsewhere in the body is tuberculosis of the spleen. It may produce a picture simulating polycythemia vera or may lead to an anemia.

Malaria

Splenic enlargement resulting from malaria may persist long after the active phase of the disease has passed. A careful history must be taken in all patients with an unexplained enlargement of the spleen to exclude the possibility of a previous malarial infection.

Kala-azar

Kala-azar is a tropical disease with enlarged spleen and liver, anemia, leukopenia, and diarrhea. The diagnosis is established by finding Leishman-Donovan bodies on sternal or splenic puncture.

Still's Disease

A syndrome occurring in children, Still's disease presents anemia and splenomegaly associated with deforming arthritis.

Infectious Mononucleosis

Infectious mononucleosis causes a generalized lymphadenitis as well as splenomegaly. The diagnosis is established by the presence of many abnormal lymphocytes in the blood stream and a positive heterophile antibody reaction.

Congestive Heart Failure

An enlarged spleen is usually found at necropsy examination after congestive heart failure, but only rarely is it demonstrably enlarged on physical examination.

Cysts or Tumors of the Spleen

Splenic cysts or tumors are comparatively rare causes of splenomegaly.

Idiopathic Hypochromic Anemia

Slight enlargement of the spleen is encountered in about half of the patients having idiopathic hypochromic anemia, but there is no leukopenia or icterus.

Familial and Acquired Hemolytic Icterus

Hemolytic icterus is recognized by the characteristic features of the blood, increased fragility of the erythrocytes, spherocytosis, and leukocytosis.

Erythroblastosis Fetalis

A large spleen is present in all forms of erythroblastosis fetalis, but the age incidence, leukocytosis, and presence of many nucleated erythrocytes establish the diagnosis.

Other hemolytic anemias such as *erythroblastic anemia*, *sickle cell anemia* in its early stage, and *acute hemolytic anemia* will seldom be confused with Banti's syndrome even though jaundice, splenomegaly, and anemia are present.

Portal Cirrhosis of the Liver

Portal cirrhosis of the liver causes an increased pressure within the portal circulation and congestion of the spleen. The anemia may be macrocytic in type. It is accompanied by mild icterus, evidences of liver insufficiency, and the development of a collateral circulation to compensate for the portal obstruction.

Hypertrophic Biliary Cirrhosis

Although the splenomegaly is not great in hypertrophic biliary cirrhosis, the liver is markedly enlarged, and the jaundice is deep and persistent.

Lipoid Dystrophies

In the lipoid dystrophies there is an abnormality of lipoid metabolism, and the cells of the reticulo-endothelial system take up and store large amounts of lipoid material. The spleen becomes markedly enlarged because the reticulo-endothelial cells contain so much of this material.

Amyloidosis

Amyloidosis is usually found in association with a chronic suppurative lesion, commonly of the lungs or bones, but it may occur in an idiopathic form. Since the kidneys are usually involved, albuminuria and cylindruria are common findings. Renal failure and uremia are often terminal events. The kidneys are not involved in some cases, and the liver may or may not

it has been found to be considerably lower. Spontaneous vascular thrombosis is a frequent postoperative complication.

There is no specific form of therapy aside from splenectomy. Diuretics are of value in lessening the ascites. In our experience mersalyl with theophylline has been the most effective preparation for use in such cases. A high protein, high carbohydrate, and low fat diet, such as is used in other types of liver disease, is of value in those cases in which hepatic damage is the underlying cause. Such a diet will contain from 125 to 200 Gm. protein, 350 to 400 Gm. carbohydrate, and about 50 Gm. fat. It is obvious that such a diet would be rich in naturally occurring vitamins but in spite of this fact additional vitamins are added, usually in the form of one of the multivitamin capsules. This is frequently augmented by the administration of liver extract although the rationale for this is not entirely clear. In addition to this diet choline citrate is given, either 20 cc. of a 25 per cent solution or 10 cc. of a 50 per cent solution three times daily, or methionine 2 Gm. per day.

An iron salt will hasten hemoglobin regeneration in those patients who have hemorrhaged but the anemia of Banti's syndrome without hemorrhage does not respond to either iron or liver extract.

Injection of the esophageal varices by a sclerosing agent through an esophagoscope has been tried in an attempt to lessen the danger of rupture and hemorrhage. Other varicosities develop, however, and repeated injection of the newly formed varicosities is necessary. It is difficult at the present time to evaluate the benefits derived from this procedure.

BANTI'S SYNDROME—SYPHILITIC

Banti's syndrome may be produced by a variety of pathologic lesions among which is syphilis, of either the acquired or the congenital type. The clinical features of the syphilitic form of Banti's syndrome do not differ significantly from those encountered when the disease is produced by other means except for the positive serologic reactions and other manifestations of syphilis which may be present. It deserves particular attention, however, because of its frequency and because of certain differences in therapy.

The pathologic alterations encountered in the liver are of particular interest. Sclerogummatous lesions are common and produce the so-called "hepar lobatum" in which the liver is reduced in size and its surface so fissured and lobulated by linear and stellate scars that it is almost unrecognizable in its gross appearance. The liver tissue may be largely displaced by

Infections

Many types of infectious diseases are associated with enlargement of the spleen, particularly those in which there is invasion of the blood stream and a chronic course. Undulant fever, typhoid and paratyphoid fever, and subacute bacterial endocarditis present splenomegaly as a prominent feature of their clinical picture.

Treatment

Splenectomy is the advisable and most effective form of treatment in Banti's syndrome, particularly in the early stages of the illness. In patients in whom an obstruction of the splenic vein is at fault the procedure results in complete cure. The results are less satisfactory in cases which are a result of portal cirrhosis, particularly if the operation is not performed until ascites has made its appearance. If the syndrome is a result of portal cirrhosis, the hepatic damage will be evident by the time symptoms are severe enough to justify splenectomy; if cirrhosis of the liver is not present at that time, it will not develop subsequently. Removal of the spleen in patients with cirrhosis of the liver lessens the volume of blood in the portal circulation and reduces the pressure within that circuit to such an extent that the danger of a ruptured varix is lessened. Because of this reduction in the pressure within the portal circulation and possibly through the development of a collateral circulation in the resulting scar tissue there is a lessening in the rate at which fluid collects in the abdomen. Permanent relief from the ascites accompanying cirrhosis of the liver has been reported after splenectomy. The beneficial results of splenectomy therefore range from complete cure, when there is mechanical blockage of the splenic vein, to varying degrees of relief from the ascites, jaundice, anemia, and leukopenia. The dragging sensation resulting from the weight of the enlarged spleen is relieved.

The results of splenectomy are frequently unsatisfactory and the course of the disease may be unaltered by the operation. Howell does not advise splenectomy since he did not find any significant difference in the course of the disease when a group of splenectomized patients was compared to a group not subjected to the operation. Others share this view. It is difficult to evaluate the statistics from various clinics as to the efficacy of the operation but in the author's opinion, as expressed in the preceding paragraph, the operation is advisable if evidences of liver insufficiency have not progressed too far. The immediate postoperative mortality has been reported to be in the neighborhood of 10 per cent but in other groups of carefully selected cases

the arsenicals themselves may cause liver damage, they must be given cautiously. It may be necessary to restrict the antisyphilitic therapy to bismuth, mercury, and iodides.

The best results are obtained when treatment is started in the early stages of the disease. In some patients this will bring about a reduction in the size of the spleen to near normal and the disappearance of other symptoms. In others the results are less satisfactory and splenectomy may be advisable. The effects of this operation are similar to those obtained in patients in whom the disease is not due to syphilis but the operation should always be preceded by an adequate course of antisyphilitic therapy.

FELTY'S SYNDROME

A rare symptom complex which is similar or closely related to Banti's syndrome was described by Felty in 1924. It consists of anemia, leukopenia, and splenomegaly with the additional features of chronic arthritis of the rheumatoid type, a yellowish brown pigmentation of the exposed portions of the skin, and, in some patients, a generalized lymphadenopathy. The late stages of the disease may be characterized by ascites and jaundice and other evidences of hepatic insufficiency. Hematemesis may occur at any time during the course of the illness.

In most respects the condition is similar to Banti's syndrome with the added features of a chronic deforming arthritis and a more profound leukopenia. In the patients observed in this clinic the leukocyte counts have been found to be in the neighborhood of 1000 to 1500 rather than around 3000 to 4000 as is true in the typical case of Banti's syndrome. Felty's syndrome appears later in life than does Banti's syndrome, being most common in middle or late adult life whereas Banti's is usually encountered in younger individuals.

The status of this syndrome as a distinct entity has been the subject of considerable controversy. It has been considered a counterpart of Still's disease, affecting adults rather than children, although Still's disease is usually accompanied by a normal or elevated leukocyte count rather than a leukopenia. Another view holds that it is merely an example of Banti's syndrome with a concomitant rheumatoid arthritis which in itself may cause a mild anemia, leukopenia, and a splenomegaly. A question of its relationship to an infectious process and sepsis lenta due to *Streptococcus viridans* has also been raised. Whether it is entitled to separate consideration is certainly open to question.

scar tissue or by gummatous infiltration, but the remaining portions of liver tissue undergo hyperplasia to compensate for that which was destroyed (Fig. 46). Thickening of the capsule of both the liver and the spleen is almost always encountered. There may be such a mass of scar tissue around these organs that removal of the spleen becomes an exceedingly difficult surgical procedure.

Syphilitic involvement of the spleen may occur in either the acquired or the congenital type of infection and may reproduce the clinical picture of Banti's syndrome without there being any obvious involvement of the liver

Treatment

An intensive course of antisyphilitic treatment is indicated in patients in whom syphilis of the liver or spleen is responsible for the production of



FIG 46 Transverse section of the liver from a case of syphilitic hepatitis producing Banti's syndrome. This shows the extensive scarring, especially about the hilus and throughout the shrunken right lobe (Korns, *Am J M. Sc.*)

Banti's syndrome. Treatment should consist of the concomitant administration of potassium iodide and either bismuth or mercury. Intramuscular injection of an insoluble bismuth-in-oil preparation at weekly intervals or semiweekly injections of a water-soluble preparation should be given. Potassium iodide in a dosage of 15 to 30 grains three times daily may be given at the same time. One of the arsenical antisyphilitic remedies may be tried after a two-month course of treatment with bismuth but should be used with caution and in small amounts, never exceeding the equivalent of 0.3 Gm of arsphenamine per week. If the arsenicals are well tolerated, a continuous course of treatment of at least two years' duration is advisable, using bismuth and arsenical preparations during alternating six-week periods. Since

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When splenectomy has been performed on patients with Felty's syndrome, transient relief from the symptoms has been obtained. The results of the operation are not as encouraging as in the ordinary type of Banti's syndrome. Pathologic features indistinguishable from those encountered in Banti's syndrome were found in one patient with Felty's syndrome who died of mesenteric thrombosis following splenectomy in this clinic. There were evidences of portal cirrhosis and of increased pressure in the portal circulation as shown by a marked dilatation of the splenic and mesenteric veins.

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tion. Improvement is rapid when adequate fluids are retained and the blood volume is brought to normal. There is also hemoconcentration in severe shock, which results from the redistribution of body fluids.

The spleen acts as a storehouse for erythrocytes. Such stimuli as exercise, emotional disturbances, and epinephrine cause it to contract and expel additional erythrocytes into the circulation. A slight transient polycythemia results.

SECONDARY POLYCYTHEMIA—ERYTHROCYTOSIS

Secondary polycythemia represents an actual increase in the number of erythrocytes and in the amount of hemoglobin as well as an increase in their concentration per unit volume of blood. The polycythemia is secondary to some condition which acts as a stimulus to erythropoiesis. A lowered oxygen tension in the blood has been shown to have such a stimulating effect and is probably one of the important factors in regulating red cell production. An abnormally low oxygen tension in the blood will be present at high altitudes because of the low oxygen pressure in the air. It will also result from cardiocirculatory disturbances and with any lesion which interferes with the gaseous exchange in the lungs.

Causes

High Altitudes

It has been recognized for many years that exposure to the low oxygen pressures which prevail at high altitudes results in an increase in the erythrocyte count and in the hemoglobin level. There are apparently two stages in the development of this polycythemia. The first reaction appears rapidly during ascension from low to high altitudes, and the greater number of erythrocytes is unassociated with any evidence of new cell formation. It is probably the result of contraction of the spleen with expulsion of the reserve supply of erythrocytes into the blood stream. The second stage appears after the individual has remained in the area of low barometric pressure for some days. It is accompanied by evidences of new cell formation and is presumably due to the anoxic stimulation of the erythropoietic centers in the bone marrow. The height of the response is reached after about one week's exposure to the low barometric pressure, and the polycythemia persists as long as residence in the high altitude continues. Polycythemia is present in natives who are accustomed to the high altitude as well as in newcomers; counts as high as 7.5 to 8 million have been found in the Indians of the Peruvian Andes.

POLYCYTHEMIA

POLYCYTHEMIA IS A CONDITION CHARACTERIZED BY AN INCREASED NUMBER OF erythrocytes and an increase in the amount of hemoglobin per unit volume of blood. The average number of erythrocytes in the normal adult ranges from 4.5 to 5 million cells per cubic millimeter of blood although counts of 6 million with a correspondingly high hemoglobin level are not infrequently encountered in active healthy males. In a consideration of polycythemia it is essential to distinguish between those erythrocyte and hemoglobin values which represent a high normal level, those which represent a physiologic increase, those in which the increase is secondary to some pathologic condition causing a demand for additional erythrocytes and hemoglobin, and those in which there is an idiopathic hyperplasia of the bone marrow—polycythemia vera or erythremia. Conditions in which there is an increased stimulus to hematopoiesis, whether secondary or idiopathic, have not only an increase in number of erythrocytes and hemoglobin per unit volume of blood but also an increase in the total blood volume.

RELATIVE POLYCYTHEMIA

Relative polycythemia refers to that condition in which the erythrocyte count and the hemoglobin per unit volume of blood are increased although there is no increase in the actual number of cells or amount of hemoglobin. It is encountered in patients who have lost large amounts of fluid from the body, or whose fluid intake is markedly restricted, and represents a diminution in the volume of blood plasma and in the total blood volume with a resultant hemoconcentration. The amount of hemoglobin and the number of erythrocytes per unit volume of blood are increased without an absolute increase in either of these. Such a condition is found in severe burns, persistent diarrhea, or severe vomiting and may be present to a slight extent with the polyuria of diabetes mellitus. The patient shows evidences of dehydra-

Acquired Heart Disease

Some forms of acquired heart disease are occasionally associated with polycythemia, but it is seldom as severe as that found in the congenital form. Mitral stenosis, particularly when it develops early in childhood, is the lesion most prone to produce such changes.

Chemicals and Drugs

Certain chemicals and drugs may produce polycythemia. Among these are arsenic, phosphorus, cobalt, aniline dyes, and nitrobenzol. Chronic carbon monoxide poisoning may also cause a moderate degree of polycythemia.

POLYCYTHEMIA VERA (ERYTHREMIA)

Polycythemia vera is a chronic progressive disease of unknown cause characterized by an increase in the number of erythrocytes, the amount of hemoglobin, and the total blood volume and by hyperplasia of the hematopoietic bone marrow. The outstanding clinical feature is the dusky, brick-red color of the skin and mucous membranes.

The term *erythremia* has been applied to the disease in recent years in contradistinction to the term *erythrocytosis*, which is used to denote a transient or secondary increase in the number of erythrocytes. In this way the terms are similar to the use of leukemia and leukocytosis in reference to changes in the white blood cells. The disease is also known as Vaquez's disease because of the original description written by Vaquez in 1892. Since Osler called attention to this disease in two papers published in 1903 and 1904, it has also been termed Osler's disease.

Incidence and Etiology

Polycythemia vera is a relatively rare condition although recent reports have stressed the great variety of clinical manifestations. They have suggested that many cases are overlooked and that its actual incidence is considerably greater than has heretofore been supposed. Reports by Reznikoff and others indicate that it is especially common among Jews, particularly those from eastern Europe. In a series of 134 collected cases he found that 48 per cent occurred in patients of Jewish descent. All of the 19 cases forming the basis for a report by Dameshek occurred in Jews. On the other hand there were no Jewish patients in a series of 26 cases from the University of Iowa clinic. The disease is extremely rare among Negroes.

Polycythemia vera is more common in men than in women and is most

The increase in hemoglobin parallels the increase in number of erythrocytes so that a normal color index is maintained.

Chronic Pulmonary Disease

Chronic disease of the lungs such as fibrosis, emphysema, or lesions of the pulmonary vascular bed may interfere with the gaseous exchange between the alveoli and the pulmonary circulation. The interference may be great enough to cause a lowered oxygen tension in the blood and a resultant anoxia of the bone marrow which stimulates the formation of erythrocytes.

Emphysema. Emphysema is the most common of the pulmonary lesions which cause polycythemia although a greater increase in the erythrocytes is apt to occur when there is thickening of the walls of the pulmonary vessels.

Ayerza's Disease. Ayerza's disease is the most outstanding example of a lesion of the pulmonary vessels which will cause polycythemia. It is a rare disease of syphilitic origin characterized by a thickening of the walls of the pulmonary vessels, extending into the smallest of the capillaries. There is an interference with gaseous exchange in the alveoli because of this fibrosis so that the oxygen tension of the blood is markedly lowered. This acts as a stimulus for an accelerated production of erythrocytes. Features of the disease are polycythemia, cyanosis, shortness of breath, and hypertrophy of the right ventricle. Evidences of failure of the right ventricle of the heart ultimately develop. The term "Ayerza's disease" is sometimes loosely used to include almost any condition characterized by extreme perivascular and peribronchial fibrosis of the lungs when associated with polycythemia. It seems better to restrict its use to the specific syphilitic entity rather than as an all inclusive term.

Congenital Heart Disease

Those types of congenital heart disease associated with cyanosis are prone to produce polycythemia. Such lesions usually have a defect which allows a shunting of the blood from the right side of the heart or pulmonary artery into the arterial circulation. In this way unoxygenated blood is carried to the tissues, and the lack of oxygen in the bone marrow acts as a stimulus to erythropoiesis. Among the congenital defects which may produce polycythemia are pulmonary stenosis with a defective septum, the tetralogy of Fallot, and complete transposition of the arterial trunks. Patients with these defects usually have clubbing of the fingers and toes, retardation of growth, and varying degrees of cardiorespiratory distress in addition to cyanosis and polycythemia.

found in this organ. The liver is likewise enlarged and hyperemic and may contain islands of hematopoietic tissue.

All organs of the body show extensive degrees of hyperemia and engorgement of the capillaries. Thrombosed vessels and anemic infarcts are not uncommon. Hemorrhages into the various organs or beneath the serous surfaces are frequently encountered. Peptic ulcer is found in about 10 per cent of the patients. A fibrosis of the vascular system within the bone marrow has been described by Reznikoff.

Symptomatology

The onset of polycythemia vera is so insidious and the early symptoms are so vague and indefinite that it is difficult to ascertain exactly when the disease begins with any degree of accuracy. It is probable that the incipient or developmental phase requires several years to reach its peak, but, as Rosenthal and Bassen have emphasized, there are no hematologic features on which to base the diagnosis during this stage. The condition cannot be recognized before the actual polycythemic stage has developed.

In many instances the disease is practically asymptomatic and is discovered on a routine blood examination. More frequently the patient complains of headache, dizziness, a feeling of fulness in the head, mild confusion, weakness, tiredness, or shortness of breath. The symptoms are frequently vague and ill defined and may be referred to almost any organ or system of the body.

The primary feature of the disease is the great increase in the hemoglobin, erythrocyte count, and total blood volume, which leads to engorgement of the entire vascular system. This engorgement and the resultant sluggish peripheral blood flow is responsible for the peculiar color imparted to the skin and mucous membranes. Since the same degree of engorgement is present in all the organs, the symptoms may be referable to any part of the body.

Skin and Mucous Membranes

One of the most characteristic and outstanding features of the disease is the dusky, brick red color of the skin, which is most apparent about the face; on the nose, ears, lips, cheeks, and neck. It is present but less striking on the distal portions of the extremities. The dusky red color is not a true cyanosis although a bluish tint may occasionally become apparent. Cyanosis is readily induced in a patient with polycythemia because of the elevated hemoglobin concentration. The peculiar color depends upon the intense engorgement and distention of the superficial capillaries. The sluggishness of the circulation allows a greater degree of oxygen loss than is normal in the

frequently encountered in middle or late adult life. The youngest patient in our series of cases was 36 and the oldest 70 with an average age of 54.6 years. There have been several reports on the familial incidence of polycythemia vera. Undoubtedly such a familial form exists although the question has been raised as to whether it is identical to the nonhereditary variety. The age of onset is much lower in the familial cases so that the disease frequently appears in early childhood and its course is more benign, being almost asymptomatic in many instances.

The cause is unknown. Evidence is lacking to support the hypothesis that there is a decreased rate of destruction of the erythrocytes or that the life span of the erythrocytes is increased beyond normal. An increased production of cells appears to be the basic feature of the disease although the cause for this has not been ascertained. It is commonly believed that the disease is similar to leukemia and represents a malignant or neoplastic type of hyperplasia of the erythropoietic bone marrow. Reznikoff believes that it is caused by anoxia of the bone marrow which results from fibrosis and thickening of the walls of the capillary vessels within the marrow so that the blood is unable to give up its oxygen to the tissues. The anoxia acts as a stimulus for the production of erythrocytes. The cause for such a fibrosis in the capillaries is not known, but the similarity to thromboangiitis obliterans was suggested. Further histologic studies are necessary to substantiate this view.

An endocrine dysfunction has been proposed as the cause, but there is no proof to support this contention. It has also been suggested that an excessive secretion of the gastric hematopoietic factor might be the cause. This view is disproved by the fact that polycythemia cannot be produced by the administration of excessive amounts of liver extract, nor can it be cured by repeated gastric lavage.

Pathology

The bone marrow is dark red in color. Microscopic examination reveals hyperplasia and increased cellularity. All of the cellular elements of the marrow are increased, but the erythropoietic cells predominate. Megakaryocytes and leukopoietic cells may be especially prominent in some cases. The cellular portion of the marrow is increased in its extent so that it encroaches upon that portion which is normally filled with fatty tissue. The picture is that of hyperplasia with extension of the hematopoietic marrow beyond its usual confines.

The spleen is enlarged, firm, and deep bluish red in color and is found to be engorged and distended with blood. In some cases hematopoietic foci are

eral vessels as well. Thromboangiitis obliterans may occur and present the usual clinical manifestations and pathologic changes, but many patients complain of painful extremities, both on rest and on exertion, without the typical pathologic features of this disease. Warm, painful, and sweating extremities are not infrequently encountered. Erythromelalgia and Raynaud's disease may also be simulated.

Neurologic Symptoms

Symptoms referable to the central nervous system are probably the most frequent manifestations of the disease and are a result of the sluggishness of the cerebral circulation. Headache and dizziness are the two most common complaints. A sense of fulness in the head and a slight sense of confusion may also be noted by some patients. Others who have noticed no complaints of this nature are aware, after treatment, that their head seems more clear and that a sense of pressure has disappeared. Weakness, fainting spells, tinnitus, poor memory, mental depression, irritability, inability to concentrate, numbness and tingling of the extremities are only a few of the cerebral and neurologic symptoms which may occur. Scotomas, dimness of vision, and spots before the eyes are common ocular manifestations.

More serious are the vascular accidents which are frequently the cause of death. Cerebral hemorrhage and progressive thrombosis of cerebral vessels result in hemiplegia, aphasia, or other types of neurologic syndromes depending upon the area involved.

Hemorrhages from mucous membranes are frequent, occurring either as slight oozing or as profuse hemorrhages with the loss of large amounts of blood.

Physical Examination

The outstanding feature on examination is the peculiar dusky red color of the skin and mucous membranes. Hemorrhages into the skin or mucous surfaces are frequently encountered. The eyes are usually congested, bloodshot, and lacrimating profusely.

The spleen is enlarged in a majority of the patients and was palpable in 19 of our 26 cases. It is firm and smooth, retaining its normal configuration. The enlargement may not become apparent until late in the course of the disease, and the size varies from an organ which is barely palpable to one which extends to the brim of the pelvis. Infarcts of the spleen are of common occurrence and cause severe pain. A friction rub may be audible over the splenic area because of the perisplenitis.

peripheral vessels, and a slight degree of cyanosis results. Dilated capillaries in the skin may become prominent in addition to the diffuse red color. Small hemorrhages in the skin or large ecchymotic areas may appear. Various types of skin eruptions are common.

The color of the mucous membranes is similar to that of the skin, the deep red color being apparent on the lips and conjunctiva. The eyes may be slightly injected, and lacrimation is frequently excessive. Hemorrhages from the nose and gums are common.

Gastrointestinal Tract

Some type of gastrointestinal disturbance is present in a majority of patients with polycythemia vera because of the vascular engorgement throughout the intestinal tract. This may be mild, consisting only of dyspepsia with gas and bloating, or it may be severe and distressing. Peptic ulcer with its usual symptoms has been found in about 10 per cent of the patients. Hemorrhages of varying severity into the stomach or bowel may occur as a result of the intense congestion. Thrombosis of the mesenteric vessels is not infrequent and produces severe pain suggestive of peritonitis.

Cardiovascular System

Symptoms suggestive of cardiac disease are frequently encountered in patients with polycythemia vera and were present in 12 of our 26 patients. Shortness of breath on exertion is the most frequent of these, but paroxysmal nocturnal dyspnea, palpitation, substernal pain or pressure, and dependent edema may occur. Cardiac hypertrophy is not common in uncomplicated cases, and a severe grade of polycythemia may be present without evidence of cardiac disease. However, since it occurs in late adult life, polycythemia vera is frequently associated with arteriosclerosis, hypertension, and cardiac hypertrophy. It is difficult to evaluate the part played by the polycythemia in producing these vascular changes. Thrombosis of a coronary artery with the usual picture of coronary occlusion is not an infrequent complication and may be the terminal event.

Peripheral vascular disease of various types occurs in a large percentage of patients. The frequency with which thromboangitis obliterans appears as a complication of polycythemia vera has been commented upon by several observers. The increased viscosity of the blood, the sluggishness of the circulation, and the rise in the number of platelets all predispose to spontaneous intravascular thrombosis. This accounts not only for thrombosis of the coronary, cerebral, and mesenteric vessels but for thrombosis of the periph-

A leukocytosis of variable degree is frequently but not consistently present. In 21 of our 26 cases the leukocyte count was 10,000 or over, the highest being 40,000. A majority of the counts ranged between 12,000 and 20,000, but much higher values are occasionally encountered. The leukocytes vary in degree of immaturity, and there is an increased percentage of nonsegmented neutrophils. A few myelocytes (2 to 3 per cent) are occasionally found. The higher total count is due to an increase in the granulocytic series of cells. Eosinophils and basophils may be slightly more abundant than normal.

The platelets are frequently more numerous, as evidenced in just 50 per cent of our cases. Extremely high counts have been recorded by some observers, and by the volumetric method we found values of over 2 per cent in 5 of the cases, 0.6 per cent being the upper limit of normal. This increase in the number of platelets and in the leukocyte count indicates that the entire cellular portion of the marrow is hyperactive and that the hyperplasia is not confined to the erythropoietic elements.

The bleeding time and coagulation time are normal. The clot frequently retracts poorly, and the sedimentation rate is decreased. More erythrocytes are being destroyed than normal, which produces an increased bilirubinemia, an elevated van den Bergh reaction and icterus index, and an increased amount of urobilinogen in the feces. The plasma proteins are not significantly altered.

The sternal bone marrow is hyperplastic with the hypercellularity involving all of the marrow elements so that the erythrocytic-myeloid ratio is not significantly altered. Many nucleated erythrocytes in various stages of development are found but myelocytes and myeloblasts are likewise a prominent feature.

Although the basal metabolic rate may be increased, this is not a constant finding. The urine commonly shows small amounts of albumin and a few casts, but hematuria resulting from hemorrhage into the urinary tract is rarely encountered. It has been shown that the cardiac output is normal in patients with polycythemia vera. In the absence of hypertension the cardiac work is within normal limits. The venous pressure is normal, but the capillary blood flow is greatly reduced because of the increased blood volume and vasodilatation.

There have been numerous reports of the disease terminating with a hematologic and pathologic picture of myelogenous leukemia so that an intimate relationship between the two diseases has been assumed. In other instances there is a terminal drop in the hemoglobin and erythrocyte levels so that a severe anemia supervenes.

The liver is usually enlarged to a slight degree, but jaundice is not common. Examination of the ocular fundi reveals engorgement of the vessels, increased tortuosity, and irregularity of their caliber. Papilledema is occasionally noted. The heart may be enlarged, and the blood pressure is elevated in many of the patients. Reflex and sensory changes in the extremities appear when lesions in the central nervous system, such as cerebral hemorrhage or thrombosis, are present.

Hematologic Features

The erythrocyte count is increased, frequently to 8 or 9 million and sometimes to 11 or 12 million erythrocytes per cubic millimeter of blood. The average size of the cells may be slightly reduced. Wintrobe found the average mean corpuscular volume to be 80 cubic microns as compared to the normal of 87.

The hemoglobin is likewise increased. Values from 18 to 24 Gm. per 100 cc. of blood are common. Higher values have been recorded occasionally. The increase in the hemoglobin is usually somewhat less than the increase in the number of erythrocytes so that there is a slightly lowered color index and mean corpuscular hemoglobin. The erythrocytes on a blood smear appear essentially normal with but slight or no evidence of hypochromia. Some polychromatophilia and basophilic stippling may be noted as evidences of the rapid production of erythrocytes. The number of reticulocytes is slightly raised, and an occasional nucleated erythrocyte may be seen on the smear.

The hematocrit is markedly elevated. This increase in the volume of the packed erythrocytes is one of the most important factors accounting for the symptoms of the disease. The reading was found to be 65 or higher in over 80 per cent of our cases, the highest value being 77. Wintrobe has found it to be as high as 84. The great increase in the volume of packed red cells explains the greater viscosity and specific gravity of the blood, and thus, together with the increased total blood volume, explains the vascular congestion and sluggishness of the circulation. The total blood volume may reach 10 liters or almost twice the normal value. Haden has shown that this increase is almost entirely due to the increased cell mass with but little change in the volume of plasma. The greater blood volume results in a marked vasodilatation, the capillaries becoming elongated, tortuous, and distended with blood. The specific gravity of the blood ranges from 1.075 to 1.080 as compared to the normal of 1.055 to 1.065, and the viscosity is from 5 to 8 times as great as normal.

Treatment

The primary object in the treatment of polycythemia vera is a reduction in the viscosity and the volume of blood since these are the features chiefly responsible for the symptoms of the disease. Numerous methods of treatment have been used. The most successful appears to be the removal of blood by venesection combined with irradiation of the erythropoietic centers so as to slow the regeneration of erythrocytes. By this means prolonged remissions may be produced.

Venesection

Removal of 500 cc. of blood at daily or two day intervals until the hematocrit and the erythrocyte count approach their normal values results in prompt relief from most of the subjective manifestations. The procedure may be repeated as often as is necessary to maintain an approximately normal hematologic picture. The frequency will vary from one patient to another. Difficulty may be encountered in drawing blood at the first venesection because of the increased viscosity, the sluggish flow, and rapid coagulation. This difficulty disappears after one or two bleedings. It is our practice to maintain the hemoglobin and hematocrit levels at a point slightly above the normal values rather than to bring them down to normal. There is no contraindication to the use of this blood for transfusion purposes. We are accustomed to administer it to any patient requiring whole blood transfusions.

Repeated blood loss results in a reduction of the iron reserves of the body and a consequent slowing in the rate at which hemoglobin and erythrocytes are being formed. What is essentially an iron deficiency anemia develops in these patients. A low iron diet has been advocated in conjunction with the bleedings, but this is not necessary as the blood loss alone will lower the iron reserves enough. It is advisable, however, to prohibit the eating of liver and other foods which Whipple has shown to be most effective in hastening hemoglobin regeneration.

Venesection alone may be used in the treatment of polycythemia vera but is best used in conjunction with irradiation therapy.

Irradiation

Irradiation may be used alone, but since its effects are slow in appearing and there is no immediate relief of symptoms, it is best combined with venesection. It may be given at the same time or after the blood is removed. It is applied over those bones which are active in erythropoiesis—the sternum, ribs,

Diagnosis

The symptomatology of polycythemia vera may be vague and indefinite or it may suggest the presence of peripheral vascular, cardiac, or gastrointestinal disease. The diagnosis is based more on the characteristic laboratory features and the physical findings than on the symptomatology. The important diagnostic points are: (1) the increase in the hemoglobin level, (2) the increased hematocrit and blood volume, (3) the elevated erythrocyte count, (4) elevated leukocyte and platelet counts, (5) enlargement of the spleen, and (6) the characteristic color of the skin and mucous membranes. Evidences of accelerated activity of the bone marrow are shown by the polychromatophilia of the erythrocytes and the slight immaturity of the leukocytes.

Secondary polycythemia or erythrocytosis must always be excluded. In most instances this can be done by excluding the presence of any primary disease of the heart or lungs which might lead to a demand for an increased oxygen-carrying capacity of the blood. The oxygen saturation of the blood is less in secondary polycythemia than in polycythemia vera, and the evidences of bone marrow hyperactivity, such as the presence of nucleated erythrocytes, leukocytosis, and increased numbers of platelets, are usually lacking in the secondary form. The erythrocyte count, hematocrit, and total blood volume seldom reach the exceedingly high values encountered in the primary form although in exceptional cases they may do so.

The early stage of polycythemia is difficult to recognize. The diagnosis is usually questionable if the erythrocyte count is below 7,000,000. A period of observation may be required before a conclusive diagnosis can be made. Tuberculosis of the spleen may reproduce the picture of polycythemia vera.

Course and Prognosis

The course of polycythemia vera is chronic. A majority of the patients have noted mild symptoms for several years before consulting a physician. A patient may live for ten or fifteen years after the onset of symptoms, but the disease is ultimately fatal and is incurable. In some instances its course is more rapid and death may occur within four or five years of the onset. As the resistance of the patients is low, the disease may terminate with an intercurrent infection. Although hemorrhages are frequent and occasionally severe, they are seldom fatal. Thrombosis of a cerebral artery or coronary thrombosis is frequently the cause of death. These may occur at any time.

but further study and longer periods of observation are necessary to evaluate radiophosphorus and to compare its value with roentgen irradiation

Radiophosphorus has also been used in the treatment of myelogenous leukemia, lymphocytic leukemia, Hodgkin's disease, and lymphosarcoma but with the exception of chronic myelogenous leukemia the results in this group of diseases have not been satisfactory.

Nitrogen Mustards

The nitrogen mustards (see p. 374) have also been tried in polycythemia vera with encouraging results but they do not seem to be as effective as either radiophosphorus or venesection combined with irradiation.

Drugs

Phenylhydrazine hydrochloride, 0.1 Gm. per day or every other day, may be given by mouth until the erythrocyte count begins to fall. It should then be given at less frequent intervals and a maintenance dose determined. The effect of the drug continues for several days after its administration has been discontinued so that the blood must be carefully and frequently studied. Administration should be stopped before the desired erythrocyte level is reached. Acetylphenylhydrazine may be used in the same dosage as the hydrochloride. It is somewhat less toxic. These drugs cause hemolysis of the erythrocytes so that evidences of excessive red cell destruction become apparent, and jaundice occasionally results. Overdosage causes anorexia, nausea, vomiting, diarrhea, jaundice, and weakness. Hematuria has been reported and an increased tendency to thrombosis. There is a cumulative action of the drugs, and a severe anemia may be produced by their continued administration. They are contraindicated in the presence of advanced arteriosclerosis or in patients who are bedfast. Satisfactory therapeutic results can be obtained by the careful use of these drugs, but their toxic effects and the danger of overdosage are so great that they are being replaced by other modes of therapy

Gastric lavage at frequent and regular intervals to remove the intrinsic factor has not been successful. Irradiation of the pyloric portion of the stomach to inhibit the formation of this substance has likewise been ineffective. Total thyroidectomy, to produce anemia or myxedema, has been tried but must be condemned. Splenectomy is harmful.

pelvis, vertebrae and the proximal ends of the long bones. The amount of irradiation should not be large since depression rather than complete suppression of the hematopoietic function is desired. It is our custom to give relatively small amounts, to observe the rapidity with which the blood regenerates, and to give further therapy when necessary. The spleen, as the organ primarily concerned with the destruction of erythrocytes, should be protected against irradiation, for it is unwise to interfere with this function.

Spray therapy or total irradiation has been advocated in recent years. It consists in the administration of 30 to 50 roentgens over the entire body from a distance of 2.5 meters at daily intervals. This method may be used in place of local irradiation, which is applied directly to specific areas. Care must be taken in any form of irradiation to prevent a severe depression of the leukocytes or the development of anemia. It must be remembered that the effects of irradiation are not immediately apparent but continue for some time after cessation of therapy.

Radioactive Phosphorus

Radioactive phosphorus which may be given orally or intravenously has been used in the treatment of this condition and has produced very promising results. Isotonic solutions of dibasic sodium phosphate prepared with radioactive phosphorus (P^{32}) may be given intravenously. The P^{32} is utilized by the body in the same manner in which ordinary phosphorus is metabolized and high concentrations are found in the bones as well as in liver, spleen, and lymph nodes. Its effect is due entirely to the emission of beta rays as the atom disintegrates and its effect is similar to that of roentgen irradiation. The half life of P^{32} is 14.3 days so that a substantial radioactivity is maintained for days. Reinhard *et al.* gave 3.5 to 4 millicuries by intravenous injection and a second injection ninety days later if the erythrocyte count was over 6 million. Occasionally this was repeated after another ninety-day period. A progressive fall in the erythrocyte count occurred with a parallel reduction in the hematocrit and hemoglobin levels. There was also a fall in the leukocyte and platelet count, sometimes to subnormal levels. The drop in the erythrocyte count does not appear until six or eight weeks after the injection because the P^{32} does not injure the circulating cells but only depresses the formation of new cells. Because of the delayed action it may be necessary or advisable to do a venesection at the beginning of treatment. With the fall in the erythrocyte count there is a subjective improvement although not complete relief of all symptoms. The spleen becomes smaller and the appearance of the patient improves. The duration of the remission varied from five to thirty-three months.

HEMORRHAGIC DISEASES

MANY OF THE DETAILS IN THE MECHANISM OF BLOOD COAGULATION REMAIN unsolved. In the absence of a complete understanding of this phenomenon there are certain to be many unexplained features in the hemorrhagic diseases as encountered in clinical medicine. A blood clot is composed of a mesh-work of fine fibrils in which erythrocytes, leukocytes, and fragments of platelets are entrapped. These fibrils are composed of fibrin, which has been derived from the soluble fibrinogen of blood plasma, and are deposited as fine needle-shaped crystals to form the basis of the clot. When coagulated blood is allowed to stand, there is a retraction or shrinkage of the clot (syneresis), and a clear slightly yellowish fluid, the blood serum, escapes. As the expressed serum is incapable of coagulation, the clotting phenomenon is obviously a function of the blood plasma rather than of the serum. Blood plasma contains prothrombin, which, in the presence of calcium and thromboplastin (thrombokinase, thrombozyme, or cephalin), is activated to thrombin. This is an active coagulant, and fibrin is formed by the reaction of thrombin with fibrinogen to form the basic structure of the actual clot.

The first step in coagulation of the blood is the conversion of prothrombin to thrombin. Prothrombin is apparently formed in the liver and is present in normal blood plasma. Although calcium is necessary for the conversion of prothrombin to thrombin, it probably acts as a catalyst, for it does not become part of the thrombin molecule. The actual activation is produced by thromboplastin. Thromboplastin is abundant in the blood platelets, but the blood plasma also contains a thromboplastic substance in its globulin fraction which is entirely separate from that found in the platelets. Cephalin is another substance possessing thromboplastic activity. Aqueous tissue extracts are rich in this material. Thromboplastin may therefore be obtained from the platelets, the blood plasma, or the tissues. There is still a question as to the method by which thromboplastin converts prothrombin to thrombin, i.e., whether thromboplastin enters into a direct combination with

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appear in crops or showers, with large numbers suddenly appearing at about the same time, so that all the lesions in one area are of approximately the same color and in the same stage of resolution. In other instances lesions of all ages may be intermingled, or large ecchymoses may occur instead of the small purpuric hemorrhages.

Purpura may occur in a great number of diseases and under a wide variety of circumstances so that the etiology is not identical in all cases. The pathogenesis in most, if not all, of the cases is an increased permeability of the capillary walls. The cause of this capillary change is not always the same. Certain cases have no recognizable etiologic factor, others are secondary to some recognized disease. Sometimes purpura is found associated with a decrease in the number of blood platelets—thrombopenic purpura, other patients present the same hemorrhagic features but have an abundance of platelets—nonthrombopenic purpura. This feature forms the basis for the most logical and the simplest of the classifications of purpura.

I Thrombopenic Purpura

- A. Idiopathic thrombopenic purpura or Werlhof's disease
- B. Secondary thrombopenic purpura associated with some other recognizable disease, intoxication, or infection

II Nonthrombopenic Purpura

- A. Idiopathic
 - 1. Schonlein-Henoch or allergic purpura
 - 2. Purpura simplex
- B. Symptomatic purpura—a miscellaneous group secondary to other diseases

IDIOPATHIC THROMBOPENIC PURPURA

Idiopathic thrombopenic purpura or purpura haemorrhagica is a disease which is characterized by purpura, by hemorrhages from the mucous membranes, and by a diminution of the platelets in the circulating blood. It was first described by Werlhof in 1735 and subsequently has been designated Werlhof's disease. The earlier literature on the subject is confusing because the differentiation between thrombopenic and nonthrombopenic purpura was not made and the primary and secondary forms were not separated. Among the important contributions to the subject were those by Bellefonds in 1811, Hayem in 1900, Frank in 1916, and Kaznelson in 1916.

The basic cause of the disease is unknown. The number of platelets in the blood stream is diminished, but the cause for their disappearance has not been ascertained, nor can it be stated with certainty that this diminution in number is the primary feature of the disease. Thrombopenic purpura is most common in children and young adults, a majority of the reported cases occur-

prothrombin, whether it acts as an enzyme, or whether it acts by neutralizing an inhibitor substance such as heparin.

Fibrinogen is converted to fibrin through the action of thrombin. The evidence at hand suggests that this is a proteolytic reaction. The fibrin is, as mentioned above, the basis of the blood clot. When the clot is first formed it is soft and spongy, but when platelets are present it shrinks, retracts, and becomes more firm. In the absence of platelets the clot remains soft, friable, and nonretractile.

Coagulation of the blood within the circulatory system does not ordinarily occur because of the presence of heparin, which prevents the conversion of prothrombin to thrombin. The mode of action of heparin is not definitely known. It may prevent coagulation either by inactivating the prothrombin itself or by neutralizing thromboplastin.

Since blood coagulation is such a complex mechanism with so many substances involved, it is theoretically possible for hemorrhagic disease to occur from a great variety of causes. A deficiency in any of the substances involved may lead to pathologic hemorrhage. It is also obvious that the pathogenesis of the recognized types of hemorrhagic disease will not be entirely clear when the basic mechanism of coagulation is so confused and so poorly understood. The hemorrhagic states can be separated roughly into three groups: (1) failure of the clot to form, owing to a deficiency of one or more of the substances concerned, (2) failure of the clot to retract and become firm, owing to a lack of platelets, (3) weakness and increased permeability of the capillary walls.

The hemorrhagic diseases comprise a heterogeneous group in so far as their pathogenesis is concerned. Although they are grouped into certain clinical entities, there are many atypical forms which are difficult to classify.

PURPURA

Purpura is a hemorrhagic disease characterized by extravasation of blood into the skin, subcutaneous tissues, and mucous membranes. The size of the hemorrhages varies, but the most characteristic lesions are small petechiae of pinhead size. The color is first a bright red but undergoes the usual changes of extravasated blood, changing from red to purple, greenish brown, and brown as the pigments are liberated. They completely disappear as the blood is absorbed. The lesions, even in the early stages, do not blanch with pressure. The term *purpura* is derived from the deep red or purple color of these small intracutaneous hemorrhages. It is common for the purpuric spots to

normal numbers in the bone marrow in most instances. The second theory, proposed by Kaznelson, suggests that the platelets are produced in normal numbers and the thrombopenia results from their excessive destruction by the spleen. In support of this theory are: the benefit derived from splenectomy by many patients with idiopathic thrombopenic purpura, the marked increase in the number of platelets in the blood following splenectomy, and the great number of platelets which are occasionally found within the spleen at the time of its removal. These two theories may not be entirely contradictory as there are some who believe that two types of thrombopenic purpura exist. In one type the disease is due to a decreased production of platelets and its course is unaltered by splenectomy. The second type is due to excessive destruction of the platelets within the spleen and is cured by removal of this organ. Dameshek has found an increased number of megakaryocytes but few platelets in the bone marrow in these cases before splenectomy whereas after splenectomy there was a great increase in the production of platelets. He believes that the disease is a form of "hypersplenism" in which the megakaryocytes are inhibited from normal platelet production possibly through a hormonal mechanism of the spleen. Definite conclusions cannot be drawn at the present time either as to mechanism by which the disease develops or as to the presence of a splenic hormone which regulates bone marrow activity. There is also conflicting evidence as to whether or not an extract prepared from the spleen of a patient with idiopathic thrombopenic purpura contains a specific platelet reducing substance.

Because of the close relationship between thrombopenic purpura, agranulocytosis, and aplastic anemia, the similarity of many of their features, and the fact that the secondary varieties of each of these diseases arise from similar etiologic agents, the three diseases have been grouped together under the term *bone marrow insufficiency*. The fact that each of the cells involved originates within the bone marrow gives credence to this correlation, and there are certain atypical, borderline cases with overlapping clinical features which cause difficulties in the differential diagnosis and further emphasize the similarities of the diseases.

Pathology

Since the histologic changes in the bone marrow and spleen in idiopathic thrombopenic purpura are not uniform, our concept of the pathogenesis has been confused rather than clarified. In some instances there is a diminution of the number of megakaryocytes in the bone marrow, or their nuclei are shrunken and pyknotic. In other cases with an equally low platelet count in

ring before the age of twenty-one. It may however occur at any age and cases during the sixth and seventh decades of life are occasionally encountered. There are many conditions to which it is secondary, possibly all cases are really secondary to some unrecognized condition, and in those which are now classified as idiopathic we are merely admitting our lack of knowledge as to possible causative agents. There are no clinical or hematologic differences between the idiopathic and the secondary types aside from modifications due to the primary disease. A familial incidence has been noted in rare instances and it may occur in a congenital form.

Pathogenesis

The pathogenesis of idiopathic thrombopenic purpura has caused much discussion and even now cannot be considered as definitely settled. It is obvious from the clinical features of the disease and from the results of the constrictor test that there is an increased permeability of the capillary walls allowing blood to escape into the skin and other tissues. Whether this increased permeability is the primary defect or whether the basic factor lies in the inability of the blood to coagulate and control the capillary leakage has not been settled. The present consensus favors the latter view. The defect in coagulation is apparently due to the absence of platelets.

It is generally agreed that the platelets are formed from the megakaryocytes in the bone marrow, have a life of but a few days or hours, and are either used up in performing their duties or destroyed by the spleen. They accelerate blood coagulation but apparently do not constitute the main source of thromboplastin. Their principal function lies in their effect on syneresis or clot retraction rather than in clot formation. Bleeding is not controlled in the absence of a firm, adherent, and retractile clot so that, although the coagulation time of the blood is found to be normal, the bleeding time is markedly prolonged. By direct observation of the capillaries under a microscope it can be seen that these vessels constrict after injury, thus helping to control the hemorrhage until the blood coagulates. The capillaries dilate to their normal size after coagulation has occurred, and if a firm clot is present there is no bleeding. If the clot is soft, friable, and nonretractile, there is bleeding when the vessels dilate.

Two explanations have been offered for the reduction in the number of platelets in thrombopenic purpura. Frank suggested that an insufficient number of platelets is produced by the megakaryocytes in the bone marrow and that the disease is essentially an insufficiency of the bone marrow. This theory cannot be proved histologically since megakaryocytes are present in

bleeding from the gums, either spontaneous or from slight trauma, are common. The gastrointestinal tract is often the site of blood loss so that the vomiting of blood or the passage of bloody or tar-colored stools is of frequent occurrence. There may be bleeding into the urinary tract with hematuria, but this is not especially common. Uterine hemorrhages are particularly frequent in women and menorrhagia is occasionally the first, and sometimes the only, manifestation of the disease. Such hemorrhages are often extremely severe and uncontrollable although the same may be true of bleeding from other areas. Vaginal bleeding before puberty is sometimes encoun-

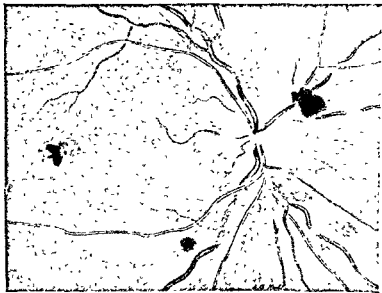


FIG. 47. Retina in a case of thrombopenic purpura showing areas of hemorrhage

tered and may be the first symptom of the disease. Hemorrhage into the joints such as occurs in hemophilia is rare. Intracranial hemorrhage is frequently the cause of death in the acute fulminating type of the disease and may also occur at any time during the course of the apparently mild and chronic forms. This may be a massive hemorrhage into the brain tissue or a subdural hemorrhage. Frequently there are multiple small but extremely widespread petechial hemorrhages. Retinal hemorrhages (Fig. 47) are very common.

Since the hemorrhage may occur from any mucous membrane and since hemorrhages into the skin are not constantly present, it is necessary to consider thrombopenic purpura in the differential diagnosis of any disease characterized by abnormal blood loss. In the acute variety of thrombopenic pur-

the peripheral blood the megakaryocytes are present in normal numbers and are structurally normal.

There is no uniformity in the histologic picture in the spleen. The findings are not specific or diagnostic. Often the spleen is essentially normal or shows a simple fibrosis. In other instances it is enlarged and shows endothelial cell hyperplasia of both the sinuses and the malpighian corpuscles. The lymphoid elements may be diminished, normal, or increased. The number of platelets found within the spleen may be very large, or they may be entirely lacking. With such a diverse histologic picture in both spleen and bone marrow one must conclude that there is no uniform pathologic change, and the possibility of there being two distinct types of pathogenesis is only speculation.

Clinical Features

Idiopathic thrombopenic purpura is ordinarily separated into acute and chronic types which differ only in the severity of their manifestations and the rapidity of their course. The acute type is more frequent in children. Its course is steadily progressive without interruptions. The chronic form usually presents less severe manifestations which may be confined to purpuric lesions of the skin. There are relapses and remissions in the hemorrhagic manifestations.

Hemorrhage into or beneath the skin, either as purpura or as ecchymoses, is the most characteristic feature of this disease and is the type of lesion from which its common name, "hemorrhagic purpura," is derived. Purpura is manifested either by a few scattered lesions or by crops and showers of innumerable small intracutaneous hemorrhages. These occur most frequently on the extremities, neck, and upper thorax but may appear on any part of the body and in severe cases may almost completely cover the body. The lesions are bright red when fresh but undergo the color changes of absorbing blood. They are not raised and do not disappear or blanch with pressure. A great number of the lesions appear in one or more areas at the same time, and after a quiescent period of variable length another shower of hemorrhages occurs. In severe forms of the disease new purpuric spots become apparent continuously rather than in showers. Purpura usually appears spontaneously but the larger and deeper ecchymotic areas are more apt to come out after slight trauma. Although purpuric lesions are the most characteristic feature of the disease, they are not always present. Severe bleeding may occur from the mucous membranes with no involvement of the skin.

Hemorrhages may occur from the mucous membranes of any part of the body. It is from this source that severe hemorrhages arise. Epistaxis and

be too low to read on the thrombocytocrit tube. The coagulation time is normal since all the elements necessary for blood coagulation are present, but because there is no retraction or firmness to the blood clot, the bleeding is not controlled and the bleeding time is prolonged. When a tourniquet is placed about the arm petechiae appear below the constriction. In mild cases there may be only a few small hemorrhagic spots, but in severe cases innumerable purpuric spots appear over the entire flexor surface of the forearm. The leukocyte count may be increased with a neutrophilic leukocytosis after a hemorrhage. Otherwise the white blood cells are not affected. The hemoglobin level and erythrocyte count are lowered as a result of the blood loss. Their levels depend upon the severity of the hemorrhage.

Variable findings have been reported from sternal aspiration and the procedure is usually of no diagnostic value except that it may exclude other conditions which might cause a secondary thrombopenia. The megakaryocytes are increased according to Dameshek, and other observers have described various abnormalities in the morphology of these cells. The other cellular elements are normal except for evidences of erythrocytic regeneration following hemorrhage.

Diagnosis

Thrombopenic purpura must be considered in the differential diagnosis of all cases of abnormal bleeding. The diagnosis rests upon the four laboratory tests mentioned plus the presence of a normal coagulation time. When the diagnosis of thrombopenic purpura is established, it is necessary to rule out the secondary form of the disease and to exclude those diseases to which the thrombopenia may be secondary before a diagnosis of idiopathic thrombopenic purpura can be made. The greater incidence of the secondary form is emphasized in the table on page 280. The frequency with which thrombopenic purpura accompanies an infection deserves special attention as it is possible for a hidden focus, such as a middle ear infection, to be the causative factor in a case that otherwise appears to be idiopathic.

Since thrombopenic purpura is an integral part of the picture of aplastic anemia, the latter must be considered in the differential diagnosis. In aplastic anemia there is a progressive anemia independent of and more severe than that produced by the hemorrhage, the reticulocytes are absent or few in number, and a leukopenia is present. In thrombopenic purpura the anemia depends upon the loss of blood, a reticulocytosis follows a hemorrhage as an evidence of erythrocytic regeneration, and the leukocytes are normal or increased in number.

pura the hemorrhage usually occurs from multiple areas and may be fatal within a short time either from exsanguination alone or as a result of a cerebral hemorrhage. The bleeding may be temporarily arrested but it usually recurs. In the chronic form of the disease purpura may be the only manifestation or it may be associated with hemorrhage of varying severity from other areas. Spontaneous remissions and relapses are the rule in the chronic cases. There may be a lessening of the bleeding tendency with advancing age, but an acute episode of hemorrhage may interrupt at any time what heretofore had been a chronic and benign course.

Fever may accompany the hemorrhagic symptoms but usually it is not high. The spleen may be slightly enlarged but this is not constant. There is no lymphadenopathy. Pallor and other manifestations of anemia will depend upon the severity of the blood loss.

A form of thrombopenic purpura has been described which is associated with multiple thrombi of capillaries and small arterioles, the thrombi apparently being composed of masses of agglutinated platelets. This has been called thrombotic thrombocytopenic purpura and thrombotic thrombocytopenic purpura and thrombotic thrombocytopenic purpura and thrombotic thrombocytopenic purpura. It is characterized by an acute febrile course, purpura, ecchymosis, and thrombopenia with a prolonged bleeding time and a nonretractile clot. A severe hemolytic type of anemia is present. There is enlargement of the liver and spleen and evidences of central nervous system involvement with headache, confusion, delirium, convulsions, muscle weakness, and coma. The leukocyte count is elevated with a leukemoid reaction of the myeloid type becoming evident. Our knowledge of this disease is based entirely on fatal cases. There is no inhibition of the bone marrow and splenectomy has been of no value. The cause of this syndrome is unknown but the outstanding pathologic feature is the pressure of the platelet thrombi.

Laboratory Features

The diagnosis of thrombopenic purpura is based primarily upon the laboratory findings. The four cardinal features are. (1) diminished platelets, (2) prolonged bleeding time, (3) nonretractile clot, and (4) positive constrictor test. The coagulation time is normal.

The platelets fall below their normal level of 300,000 to 500,000 per cubic millimeter of blood so that only 10,000 to 75,000 may be present. The critical level below which bleeding is apt to occur has been given as from 30,000 to 60,000, but there is no absolute correlation between the number of platelets and the tendency to bleed. When determined volumetrically (normal 0.4 to 0.6 per cent) the platelet level frequently drops to 0.01 or below and may

Since infections are prone to cause thrombopenia, it is possible that some cases which are apparently idiopathic in origin may actually be secondary to an infection. It is advisable, therefore, to search for and eradicate any obvious focus of infection before more radical procedures are undertaken in the treatment of the chronic form of the disease.

The chronic relapsing course of thrombopenic purpura with spontaneous remissions makes it difficult to evaluate various forms of therapy and many substances and procedures have been recommended. Among these are irradiation of the spleen, snake venom and antivenoms, liver extract, and various endocrine preparations. It is doubtful if any of these are effective.

Splenectomy

Splenectomy is the most effective remedy in idiopathic thrombopenic purpura and the advisability of this operation must be given careful consideration in every case. The procedure is of no value, and is definitely contraindicated, in secondary thrombopenic purpura so that a diagnosis of the idiopathic form of the disease must be established. When the diagnosis has been confirmed splenectomy should be done if the bleeding is at all severe and persistent and does not show evidences of diminishing in intensity after transfusions.

The operative mortality has been high when splenectomy is performed in the acute fulminating cases but it has been performed successfully even in such patients. Whenever possible the operation should be performed during a quiescent period rather than during an acute exacerbation of the bleeding. It is best to give repeated transfusions during the episode of hemorrhage in an attempt to produce a remission rather than to attempt splenectomy immediately. If the bleeding is not readily controlled by this means, it may be necessary to go ahead with the operation in spite of the active bleeding.

In many patients the manifestations are mild and consist only of purpuric skin lesions with or without slight bleeding tendencies. Splenectomy is not necessary in these mild cases especially during the first episode. If, however, there are recurrences of these manifestations it is advisable to remove the spleen. Since remissions can usually be induced by means of transfusions it is best to watch the patients through one or more attacks to determine the severity and frequency of the hemorrhagic episodes before advising that the spleen be removed. The author has seen a fatal cerebral hemorrhage occur (1) in a young girl who had had only slight purpuric manifestation on the legs, (2) in a patient who was scheduled for splenectomy the following morning, and (3) in a 70-year-old female who had only purpura and ecchymoses of mild degree for the preceding week and had never noted hemorrhagic tend-

Prognosis

In the acute forms of the disease the hemorrhage may be profuse and rapidly fatal. Even if the bleeding is controlled, the chances of recurrence are very great. Spontaneous recovery from the hemorrhagic episode is the rule in the chronic forms, but recurrences will occur in about 75 per cent of the cases although these may not appear for several months or years and will vary in their severity. Recurrences tend to be more frequent and more severe in women than in men. In some instances there may be only a single episode of hemorrhage with no recurrences. The mortality is high in those cases of thrombopenic purpura complicating pregnancy.

Treatment

Symptomatic

Local treatment may be applied to the bleeding areas when these are accessible: i.e., the use of nasal packs moistened with normal blood serum or one of the local hemostatic preparations (fibrinogen, cephalin, thrombin).

Transfusions

The most effective means of controlling hemorrhage is the administration of blood transfusions, preferably several small (150 to 250 cc) transfusions rather than a single large one. In the acute form of the disease this frequently will have no effect, but in the less severe forms, and occasionally in the acute variety, the bleeding may be completely stopped or markedly lessened. The effect is temporary and the hemorrhage is apt to recur. In our experience this has been effective in controlling the hemorrhage in a vast majority of patients with idiopathic thrombopenic purpura but has been less successful in the secondary forms. It should be used in an attempt to produce a remission in the hemorrhage manifestations before splenectomy is undertaken. Blood which has been stored in a blood bank is just as effective as fresh blood or a direct transfusion.

Intramuscular injections of whole blood, 30 to 40 cc. given in the gluteal muscles, have been effective against mild hemorrhages but are much less effective than intravenous administration.

Foreign protein injections have been tried, with questionable results. Attempts have been made to control the bleeding by sensitizing the patient to horse serum and then producing a mild reaction by subsequent injections of the serum. The results are equivocal. In our experience the procedure has not been effective.

Pernicious anemia	14
Aplastic anemia	11
Familial hemolytic icterus	1
Acquired hemolytic icterus	1
Anemia of pregnancy	1
Idiopathic hypochromic anemia	1
4. Liver disease	12
5. Miscellaneous	19

The literature on this subject indicates that the incidence of secondary thrombopenic purpura following the use of drugs is far more frequent than was evidenced in this particular series of cases.

There is no apparent factor common to all these conditions which accounts for the decrease in the number of platelets. The pathogenesis is just as obscure as in the idiopathic type. The pathologic alterations in the bone marrow and spleen are diverse and depend upon the primary disease which is present.

The clinical features are the same as those of the idiopathic variety. Hemorrhagic manifestations may be so severe as to overshadow the symptoms of the primary disease. They may be the first evidence of illness. The diagnosis rests on the laboratory features. (1) decreased platelets, (2) prolonged bleeding time, (3) nonretractile clot, and (4) positive constrictor test. The coagulation time is normal. All cases of thrombopenic purpura should be considered to be of the secondary type until the presence of a primary disease has been definitely excluded.

Causes

Infection

Infection is an important etiologic factor in secondary thrombopenic purpura. A hidden focus may account for the trouble in a patient who appears to have the idiopathic variety. We have observed several cases in which purpura appeared with each acute flare-up of a chronic infection and disappeared when the acute phase had subsided. Infection is more frequently the cause of thrombopenic purpura in children than in adults.

Toxins and Drugs

Toxins and drugs have frequently been incriminated in the production of thrombopenic purpura. The actual incidence from drugs and chemicals is much higher than that recorded in the preceding table of cases from this clinic. Among the drugs which are commonly to blame are the arsenicals used in antisyphilitic therapy, gold salts, bismuth, iodides, sedormid, benzol, dimetrophenol, and the sulfonamide group of drugs. Others, such as tridione, sodium salicylate, and pyribenzamine, have been incriminated.

encies before. After such experiences the tendency is to become more liberal in recommending splenectomy.

The operation is not successful in stopping the hemorrhagic tendencies in all cases and this fact must be presented to the patient prior to operation. There may be only a slight improvement in the bleeding tendencies and relapses may occur at variable periods after the operation. Some recurrences are due to accessory spleens which are not found and removed at the time of operation.

The platelet count increases rapidly after splenectomy reaching a peak from ten to fourteen days after the operation and then subsiding to a normal level. In the unsuccessful cases a thrombopenia may again develop after a variable period of time and hemorrhagic tendencies may again appear.

The results of splenectomy are variable. Spence reported good results in 91 per cent of the chronic and 16 per cent of the acute cases from a total of 101 patients. Whipple followed 61 patients postoperatively and found 51 with good results, 4 fairly successful, and 6 not relieved. In those patients with the acute form of the disease there were 7 postoperative deaths in 8 cases. Splenectomy is indicated in all but the very mild cases of idiopathic thrombopenic purpura in spite of the operative mortality and the fact that not all cases are cured. It is the most effective method of treatment available at the present time. There is a sudden and dramatic cessation of hemorrhage immediately after ligating the splenic vein in many patients who are operated upon during an active hemorrhagic episode.

SECONDARY THROMBOPENIC PURPURA

Secondary thrombopenic purpura is far more frequent than the idiopathic variety and is found associated with a great many diseases. The accompanying table compiled from a study of 160 cases of thrombopenic purpura shows the relative frequency of the secondary type as encountered in this clinic and the wide variety of diseases with which it may be associated.

I. Idiopathic thrombopenic purpura	17
1. Acute	3
2. Chronic	14
II. Secondary thrombopenic purpura	143
1. Infections	25
2. Toxins and drugs	6
3. Blood diseases	81
Lymphatic leukemia	18
Lymphoma	11
Myelogenous leukemia	16
Aleukemic myelosis	7

results are not as good as in the idiopathic form. The usual local measures for controlling hemorrhagé may be used.

NONTHTROMBOPENIC PURPURA

Nonthrombopenic purpura includes a number of conditions which have in common a purpuric type of skin involvement. Davis has shown that these heterogeneous conditions are actually interrelated and has found a definite familial tendency in nonthrombopenic purpura. In 27 families there were 88 cases of spontaneous ecchymoses of the skin; 79 of these were purpura simplex, 4 were Schönlein purpura, 4 were Schonlein-Henoch purpura, 2 bruised easily, and there was 1 case of pseudohemophilia. He found the manifestations appearing at progressively earlier ages in succeeding generations and also noted the frequent occurrence of rheumatic fever in the same families. This interrelation of the various types of purpura suggests that they have a common background.

In none of these conditions is there any significant alteration in the blood: The platelets are normal, the clot retracts properly, and the bleeding and coagulation times are normal. In most instances the bleeding is not sufficient to produce an anemia.

Schönlein-Henoch Purpura, Anaphylactoid Purpura

Schonlein-Henoch purpura is characterized by purpura or an erythematous type of skin lesion associated with joint or visceral manifestations. It was formerly divided into two separate types, Schonlein's purpura, in which there was involvement of the joints, and Henoch's purpura, in which there were visceral manifestations in addition to the skin lesions. The disease is rare but occurs more frequently in children and young adults than in older patients.

The cause of this symptom complex is unknown. Infection appears to be the etiologic factor in some cases, but usually an anaphylactoid or allergic reaction is strongly suspected because of the similarity of the manifestations to serum sickness. In a few instances a sensitivity to some foreign protein can be demonstrated and an attack induced by exposing the patient to this allergen. The pathologic change which is common to all cases of Schonlein-Henoch purpura is an increased permeability in the walls of the capillary vessels. This allows plasma or whole blood to escape into the skin, joints, and mucous membranes of the gastrointestinal or genitourinary tracts. Erythema, urticaria, or edema of the skin may occur as well as purpura.

Blood Dyscrasias

Blood dyscrasias accounted for the largest number of cases of secondary thrombopenic purpura in our experience. Thrombopenic purpura is an integral part of the picture of aplastic anemia, and the hemorrhagic manifestations commonly overshadow the other clinical features. It occurs in acute leukemia of all types and in the terminal stages of chronic leukemia. In aleukemic leukemia the hemorrhagic manifestations may be the predominating feature. Pernicious anemia is usually accompanied by a slight reduction in the number of platelets, but thrombopenic purpura is relatively uncommon. The other blood dyscrasias with which thrombopenic purpura may be associated are shown in the table. When this complication appears in association with another blood dyscrasia, it adds to the gravity of the prognosis as the hemorrhages accentuate the anemia and hasten the course of the disease.

Liver Disease

Various forms of liver disease frequently cause thrombopenic purpura. In many instances the number of platelets will be low, and the other laboratory features of purpura will be present even though there are few or no hemorrhagic manifestations. The occurrence of this complication, even though the hemorrhagic manifestations are not severe, adversely affects the prognosis of the primary disease.

Pregnancy

Thrombopenic purpura has occasionally been encountered during pregnancy. Although this is a rare complication, it is a serious one as the mortality has been high in both mother and fetus. Purpura has been found in the fetus of mothers having the disease.

There are many unrelated diseases to which thrombopenic purpura may be secondary. This illustrates the fact that the platelets are reduced by a wide variety of diseases.

Treatment

The treatment of secondary thrombopenic purpura is primarily that of the causative disease, but unfortunately, in many instances, little can be done for that condition. Splenectomy should not be attempted in any case in which the thrombopenic purpura is a secondary manifestation. Although transfusions will sometimes control or modify the bleeding tendency, the

rily from the train of symptoms mentioned after other types of pathologic change have been excluded. It is particularly difficult when the manifestations are confined to the gastrointestinal tract with no involvement of the skin. If an allergic state can be demonstrated, it is an aid in establishing the diagnosis.

There are no hematologic changes, the coagulation and bleeding times are normal, the blood clot is retractile, and platelets are present in normal numbers. The constrictor or arm band test is usually positive so that petechiae appear below the constriction, but this is not always true. Hemorrhage is seldom sufficient to cause a significant degree of anemia although this may occur in some cases.

Treatment

The treatment of this form of purpura is not satisfactory, consisting mainly of symptomatic measures to control the abdominal pain, diarrhea, and joint manifestations. The condition may be cured if an allergic basis can be established from the history or by skin tests for specific sensitivity and the offending food or allergen eliminated. This is usually impossible. Calcium lactate, 15 grains by mouth three times a day, or calcium chloride, 25 cc. of a 10% solution intravenously, has been effective in controlling the abdominal pain in some cases. The diet should be smooth and nonirritating when the gastrointestinal tract is involved. Belladonna or atropine may relieve the distress to some extent. Vitamins D and C have been advocated empirically, but their effectiveness has not been proved. If the skin lesions are urticarial in type, they may be controlled by the use of adrenalin hydrochloride subcutaneously, 0.5 cc. of a 1:1000 solution. If bleeding is severe it may be controlled by blood transfusions.

Purpura Simplex

Purpura simplex is a rather common type of purpura which appears in otherwise healthy individuals and is not associated with visceral, joint, or constitutional manifestations. There is no evidence to suggest an allergic background, and there are no demonstrable changes in the blood although the constrictor test may be positive. It may occur at any age; it is more common in females than in males.

Typical small purpuric spots may occur anywhere on the body but are most common on the extremities. Many of these intracutaneous hemorrhages are apt to appear suddenly. There may be a single shower of purpuric lesions or recurring showers over a period of months or years. The appearance of

Symptoms

The symptoms are extremely variable as to location, type, and severity. There are cases that exhibit only skin manifestations. These may be in the form of purpura, edema, urticaria, or erythematous lesions which may be local or generalized. The lesions usually appear suddenly and subside gradually but they may recur after an interval of a few days, after a year or more, or never. There are usually but few constitutional symptoms in instances in which the manifestations are limited to the skin although fever, chills, malaise, anorexia, and headache may occasionally usher in an attack. These symptoms are far more common if visceral manifestations are present. An acute infection such as a sore throat or upper respiratory infection may precede the skin manifestations by a few days or a week.

The articular manifestations (Schonlein's purpura) are the result of exudation of blood or plasma into the joint cavities and periarticular tissues. This causes extreme pain, tenderness, and stiffness of the joints. The clinical features are similar to those encountered in serum sickness. There is no redness or inflammatory reaction in the region of the joint and but little swelling. The joint symptoms develop rapidly in one or more joints only to subside and recur. The pain and swelling may migrate from joint to joint in much the same manner as acute rheumatic fever. Fever, malaise, and other constitutional symptoms are common in the arthritic type of purpura.

Abdominal manifestations (Henoch's purpura) occur when the extravasation of blood or plasma occurs into the walls of the gastrointestinal tract or other viscera. This may happen alone or may be associated with skin or joint manifestations. It is frequently accompanied by rather severe constitutional symptoms, particularly by fever and malaise. The abdominal pain may be severe and colicky in nature and continue for several days. It is often associated with tenderness and with rigidity of the abdominal muscles. Nausea and vomiting are common. During the acute attacks there may be gross blood in the stools, occult blood is frequently present.

The genito-urinary tract may be the site of involvement, pain, hematuria, and albuminuria being the usual manifestations. This is far less common than joint and gastrointestinal symptoms.

Involvement of skin, joints, and viscera may occur in any combination, or the symptoms may appear in any one of these locations and subsequently involve the others. Attacks of abdominal pain, vomiting, melena, and diarrhea may occur repeatedly over a period of years and the diagnosis not be suspected until skin lesions appear in association with one of the attacks.

The diagnosis of Schönlein-Henoch purpura is difficult. It is made prima-

Purpura Due to Intoxications

Purpura has been reported to have followed the use of a wide variety of chemicals and drugs. The list of those which have been incriminated would be too long to be of value, particularly since complete blood studies were not carried out in many of the reported cases. A majority of the drugs which have been mentioned are known to affect the platelets. Some cases of simple nonthrombopenic purpura are undoubtedly due to drugs or chemicals, and quinine, mercury, belladonna, and the iodides appear to be the most common causative agents. The venom of certain snakes, such as the Crotalidae (rattlesnake, copperhead, and water moccasin), causes a particularly severe form of purpura because of its toxic effect on the endothelial lining of the capillaries.

Treatment consists of removing the offending substance.

HEMOPHILIA

Hemophilia is an inherited tendency in males to bleed. It is characterized by a marked prolongation of the coagulation time of the blood although the bleeding time, as ordinarily determined, is normal. The tendency to bleed appears early and is present throughout life although it may become somewhat less severe in those reaching adulthood. The bleeding usually follows trauma rather than appearing spontaneously.

The disease and its familial tendency have been recognized for centuries. The defect is transmitted as a recessive sex-linked characteristic and follows the laws of mendelian inheritance. It affects only males, is transmitted only by female carriers who have no clinical or laboratory evidence of the disease, and is not transmitted by unaffected males. Although it is theoretically possible for a female to inherit the disease, no authentic case of this type has been reported. The possibilities of its transmission are as follows:

- 1 Hemophilic male and normal female
Sons are not bleeders and cannot transmit the disease.
All daughters are carriers
- 2 Normal male and female carrier
Sons—half of the sons are bleeders
Daughters—half of the daughters are carriers. There is no method of distinguishing the carriers from the noncarriers
- 3 Hemophilic male and female carrier.
Sons—half of the sons are bleeders
Daughters—half of the daughters are carriers and half should be bleeders although such a case has not been observed

the purpura may be accompanied or preceded by a slight tingling sensation in the involved area.

Since this condition is characterized by spontaneous remissions and relapses, the evaluation of therapeutic procedures is difficult. The use of calcium lactate or gluconate, 15 grains three times daily by mouth, has been advised and is frequently combined with the administration of vitamins D and C. The purpura has disappeared under this form of therapy although one cannot be sure that a spontaneous remission did not take place.

Symptomatic Purpura

Senile or cachectic purpura, mechanical purpura, is a simple form not infrequently encountered in elderly or arteriosclerotic persons in whom there is no evidence of disease aside from the infirmities of age. There are no alterations in the number of blood platelets or in the coagulability of the blood, and the explanation for the condition seems to be merely a capillary weakness. The lesions are apt to appear on the extremities, especially the legs. The condition is not uncommonly found in patients suffering from some wasting or debilitating disease or during the convalescent period following a severe or protracted illness. It is often seen in patients with a severe grade of malnutrition. Patients who have been confined to bed for a period of weeks, as with coronary artery occlusion, are apt to develop a few purpuric spots on the lower extremities when they first assume an erect posture. Purpura may also occur in the extremities after fractures or other injuries and also after the application of bandages or casts. No treatment is necessary and none is effective in this type of purpura.

Purpura of Infectious Diseases

Some acute infectious diseases may occasionally be accompanied by purpura. This has been noted most frequently with smallpox, scarlet fever, cerebrospinal meningitis, Rocky Mountain spotted fever, measles, typhus, and septicemia.

Purpura Due to Avitaminosis

Purpura is a part of the usual picture of scurvy, a deficiency of vitamin C. It may also appear as a result of vitamin D deficiency. Since it may be caused by chronic debility and malnutrition alone, it is difficult to evaluate the part played by the vitamins in these cases.

blood from a hemophiliac. They believe that the coagulation defect is in the plasma rather than the platelets. Patek and Stetson have shown that platelets obtained from the blood of a hemophiliac are as effective in promoting coagulation as normal platelets. Recent investigations on the globulin fraction of the plasma proteins have strengthened the belief that the defect is in the plasma and have shown that the "antihemophilic globulin" or "globulin substance" is found in subfraction II of fraction III and occasionally in fraction I of the blood plasma.

Clinical Features

The most characteristic feature of hemophilia is the tendency to uncontrollable bleeding. The bleeding may be very profuse or only a slow oozing but it is protracted and of long duration. It usually follows trauma of some type rather than appearing spontaneously, but the slightest cut, scratch, or bruise may cause a fatal hemorrhage. Although the disease is present at birth, bleeding does not commonly occur at the time of delivery or in the first few weeks of life. The tendency usually makes its appearance during infancy or childhood. Many of the patients die of hemorrhage during the first year of life. A smooth, clean incision, such as a pinprick or stab wound of the ear or finger for drawing a sample of blood, is not followed by excessive bleeding, and venipuncture may be performed with safety. The danger lies in ragged and irregular cuts or scratches in which the cut surfaces cannot be brought into apposition. Fatal hemorrhages frequently result from tooth extractions, loss of deciduous teeth or after minor surgical procedures such as circumcision.

The tendency to bleed varies from time to time. There are quiescent periods during which it is slight and patients may withstand moderate trauma without hemorrhage. The coagulation time of the blood is shortened during these stages of remission and may occasionally be found to be normal. During an active stage the coagulation time becomes greatly prolonged and hemorrhage occurs with the slightest provocation. The course of the disease is characterized by alternating quiescent and active periods of variable length and severity.

Slight trauma may produce bleeding from the mucous membranes of the nose, gums, and mouth. Hemorrhage from other mucous surfaces is infrequent. Severe gastric and intestinal hemorrhages, such as occur in thrombopenic purpura, are not common. No part of the body is exempt, however, and bleeding may occur from any area which is traumatized. Profuse hematuria has been observed repeatedly in a patient with a renal calculus.

Hemophilia is widespread throughout the world. Although it occurs predominantly in the white race, it has been observed in Negroes. The most noted cases have occurred in the Spanish and Russian royal families

The Defect in Coagulation

A delay in the coagulation time has been recognized for many years as the fundamental defect in the blood of hemophiliacs, but the exact cause for this slow coagulation has not been adequately explained in spite of numerous and extensive researches in the field. It may require many hours for a clot to form in hemophiliac blood, but once it has formed, it retracts in a normal manner and is normal in its chemical constituents and structure. The bleeding time as determined by Duke's method, in which the incision is a small clean cut which allows close apposition of the cut surfaces and relatively large amounts of tissue juice in the wound, is normal. These facts suggest either a lessening, but not a complete absence, of one of the substances concerned in the coagulation of the blood or a delayed reaction in the conversion of either prothrombin to thrombin or fibrinogen to fibrin.

Various investigators have shown that calcium, prothrombin, and fibrinogen are present in normal amounts in the blood of hemophiliac patients, that prothrombin is converted into thrombin much more slowly than normal, and that thrombin is destroyed by antithrombin almost as rapidly as it is formed. A subfraction of serum globulin obtained from normal blood produces coagulation of hemophiliac blood. The amount of this substance in the blood of a hemophiliac appears to be much less than in normal blood although equal amounts of the substance are equally effective in their coagulation-producing action regardless of their source. Blood transfusions shorten the coagulation time of hemophiliac blood. It is evident that the transfused blood supplies some factor which is lacking in the blood of a hemophiliac. Minot and Lee believe that the deficiency lies in the blood platelets. The addition of platelets from normal blood to hemophiliac plasma shortens the coagulation time whereas platelets from hemophiliac blood lengthen the coagulation time of normal plasma. Brinkhous believes that platelets from hemophiliac blood contain a normal amount of thromboplastin but liberate it more slowly than do platelets from normal blood so that the conversion of prothrombin to thrombin is delayed. These observations on the platelets support the theory that the coagulation defect in hemophilia is due to the platelets being more stable than normal and consequently disintegrating more slowly.

Pohle and Taylor have investigated the "globulin substance" from blood plasma and found it to be effective in shortening the coagulation time of

long coagulation time in vitro there is considerable sedimentation of the erythrocytes, and the clot contains but few of these cells.

The course of hemophilia varies in different patients. There are some in whom it is relatively mild; although occasional hemorrhagic episodes occur, they are not particularly frequent or severe, and the patient lives through



FIG. 48 Roentgenogram of a knee from a patient with hemophilia. Note the widening and deepening of the intercondylar notch, fringing of the margins, and cloudiness of the joint space.

the normal span of life. There is a tendency for the bleeding to become less severe with advancing age so that the outlook becomes more hopeful when the childhood years have been passed successfully. Hemorrhages are profuse and frequent in severe forms of the disease. A fatal hemorrhage during the first year of life is common. The mortality during the first ten years of life is about 40 per cent. As any trauma, however slight, is apt to result in a fatal hemorrhage, the patients live in constant fear of injury and hemorrhage.

Subcutaneous bleeding is frequent, and ecchymoses or hematomas follow a slight bruise. The pressure of clothing is sufficient to cause ecchymotic areas in severe cases. The subcutaneous hemorrhages vary in size from small discolored areas to extremely large extravasations of blood, which may be painful or may cause symptoms from pressure on nerves. Fever and leukocytosis may accompany the larger hematomas. Purpura does not occur.

Some hemophiliacs experience attacks of renal colic and pass clots of blood which are obviously blood casts. It is interesting that the blood should clot in the ureter in the absence of obstruction when the coagulation time of shed blood is extremely long. Tocantins and Lindquist have demonstrated the presence of thromboplastic activity in the urine of normal and hemophilic individuals and the clots which form are probably due to the extravasated blood coming in contact with the thromboplastin of the urine.

Hemorrhage into joints is a common manifestation of hemophilia. The large joints of the extremities are usually involved. As a result of a slight bump, jar, unusual weight-bearing, or even with no recognizable trauma the joint becomes swollen, painful, and stiff from the intra-articular bleeding. The hemorrhage may be so slight that the symptoms disappear within a few days or so profuse that the swelling and pain persist for weeks. There are frequently recurrent hemorrhages into the same joint, and fibrous adhesions result from incomplete absorption of the blood. The joint ultimately becomes stiff and ankylosed. It is uncommon to find a hemophilic who has reached adult age without one or more deformed and ankylosed joints. Roentgenograms show atrophy of bone and periosteal thickening which resemble the changes occurring in arthritis deformans (Fig. 48).

The most characteristic finding in the blood is a marked prolongation of the coagulation time. The bleeding time is within normal limits. The coagulation time varies with the severity and activity of the disease, being much longer during the active phases, when hemorrhages are most apt to occur. It becomes shorter but seldom returns to normal in the quiescent periods. The Howell prothrombin time (coagulation time of recalcified plasma) is prolonged. Prothrombin determinations by the method of Quick and Smith are normal.

The hemoglobin and erythrocytes are normal unless recent bleeding has produced an anemia. The leukocyte and differential counts are normal unless altered by a severe hemorrhage which causes a neutrophilic leukocytosis. The number of platelets in the blood is normal and they show no constant structural changes. Although the time required for coagulation of the blood is prolonged, once the clot has formed it retracts normally. Because of the

difficult to evaluate the results of therapy since spontaneous remissions occur regardless of the type of treatment.

Transfusion is the most effective procedure in controlling active hemorrhage. A single transfusion is frequently effective in the less severe cases; repeated small transfusions may be necessary for the more severe episodes. Transfusions reduce the coagulation time of hemophilic blood and so control the bleeding. The effect is transient. The maximum effect on the coagulation time is obtained immediately after giving the transfusion, and there is a gradual return to the previous level within eighteen to forty-eight hours. Small amounts of blood are just as effective but somewhat less rapid in action than large transfusions. Transfusions of 100 cc. given at six to twelve hour intervals are more effective than an equal amount of blood given in a single injection. Direct transfusion, citrated blood, stored blood from a blood bank, and blood plasma have been found to be about equally effective. Transfusions cannot be relied upon to control the bleeding in all cases. They may be of little or no benefit in patients with a severe episode of bleeding. The use of plasma has certain advantages over whole blood in that it can be stored indefinitely in a frozen state without deterioration and may be given without regard to the blood group. Extreme care should be taken to protect the veins of a hemophilic since repeated transfusions will be necessary throughout his life. A vein should never be cut and ligated for transfusion purposes.

The intramuscular injection of whole blood, 20 to 30 cc. in the gluteal muscles, has some effect in controlling mild hemorrhages but is painful and much less effective than blood given intravenously. Blood can be given by this route in case of emergency without recourse to typing or cross matching, but the procedure has no other advantage.

Surgical procedures constitute a real hazard for the patient with hemophilia and should be avoided when possible. If such a procedure is necessary, however, the bleeding can usually be controlled by means of preoperative transfusions and repeated small transfusions during the period of healing. The same precautions are necessary for the extraction of a tooth. A tooth can frequently be removed, however, by winding a short rubber band around it and forcing it against the gum margin more tightly each day until the tooth ultimately drops out without loss of blood.

Various forms of local treatment may be applied when the site of bleeding is accessible. Simple compression is ordinarily ineffectual, but the application of 1:1000 solution of epinephrine may be temporarily effective. The application of cotton or gauze soaked in normal blood serum or tissue

Diagnosis

The diagnosis depends upon the presence of a lifelong tendency to bleed in a male with a family history of a similar condition in other males on the maternal side of his family. The onset of bleeding comes in infancy; it follows slight trauma and commonly occurs into the joints. The prolonged coagulation time with a normal bleeding time are the important hematologic findings.

These characteristic features serve to differentiate hemophilia from other types of hemorrhagic disease. In thrombopenic purpura the platelets are few and the bleeding time is prolonged whereas the coagulation time is normal. Congenital fibrinopenia shows a lowered fibrinogen content of the blood. In pseudohemophilia the bleeding time is prolonged, and the family history indicates that females are affected as frequently as males. Hereditary telangiectasia is accompanied by normal hematologic findings, shows the capillary dilations, and seldom appears in infancy. Hemorrhagic disease of the newborn is associated with a lowered prothrombin content of the blood, and there is no family history of bleeding. In scurvy a dietary deficiency in vitamin C may be detected.

The detection of female carriers of hemophilia is impossible since there are no clinical or hematologic evidences of this state. The first indication of the trait is the appearance of the disease in a male offspring.

Treatment

There is no form of treatment at the present time which fundamentally alters the course of hemophilia. Recurrences of the hemorrhagic episodes will take place in spite of any of the numerous remedies which have been proposed. All possible precautions should be taken to prevent trauma of any type. The nature of the disease should be explained to the patient or his parents, and they should be warned of its dangers. The patient must lead a sheltered life and be protected against injury as far as possible. It is a common practice to carry an afflicted infant on a pillow rather than holding him in the ordinary manner and to pad the objects with which he is apt to come in contact so as to prevent bruises. Obviously the infant or child cannot be protected against all minor injuries so that hemorrhages will occur in spite of all precautions.

The treatment of active bleeding is none too satisfactory. The great number and diversity of substances and procedures which have been advocated are in themselves good indications of their ineffectiveness. It is dif-

HEREDITARY PSEUDOHEMOPHILIA

There have been numerous reports of a hereditary hemorrhagic diathesis which presents features distinctly different from those of hemophilia and thrombopenic purpura. Some of the reports refer to it as thrombasthenia although investigations have shown the platelets to be normal both in number and in their function. Platelets from a patient with this disease produce coagulation in normal platelet-free plasma. The term *thrombasthenia* therefore does not seem to be applicable, and although the term *pseudohemophilia* has many objectionable features, it is more satisfactory.

The clinical features consist of recurrent hemorrhages from the nose, gums, gastrointestinal tract, or uterus which may begin spontaneously or as a result of a minor trauma. Hematuria, hemoptysis, and hemarthrosis may occur. The bleeding may be mild or severe and is not infrequently fatal. It tends to be more profuse in younger patients and to diminish in severity with advancing age. The patients may bruise easily and bleed profusely from small cutaneous injuries, but intracutaneous hemorrhage or purpura is not common. The manifestations are present at birth or appear in infancy. Sometimes they are delayed until later childhood or adolescence.

Hematologic examination reveals a normal platelet count. The bleeding time is prolonged but the coagulation time is normal. Results of the constrictor test are variable.

The disease affects both males and females and may be transmitted by either sex directly to their sons and daughters so that the familial and hereditary features are distinctly different from those of true hemophilia. The changes in the bleeding and coagulation time are just the opposite of those found in hemophilia.

There is no satisfactory treatment. Whole blood transfusions give the best results in controlling the hemorrhage although the benefits are transient. The bleeding time is not altered by the transfusion. Injections of whole blood intramuscularly will control mild bleeding.

HEREDITARY HEMORRHAGIC TELANGIECTASIA

Hemorrhagic telangiectasia is a hereditary abnormality characterized by localized dilatation of the capillaries and venules in the skin and mucous membranes, which rupture and cause spontaneous hemorrhages. The lesions are pinhead-sized tumors composed of dilated capillaries and venules. They are bright red in color and are most frequent in the skin of the hands, face, and

extract will sometimes stop the bleeding. A globulin substance derived from human, bovine, swine, or rabbit plasma has produced good results on local application, and the same is true of purified thrombin. These may be applied to the bleeding area in liquid form on gauze, in a spray from an atomizer, or as a more forceful stream from a syringe. The powdered form is less easily washed away by the bleeding than is the liquid and is particularly effective when applied on a compression pack in a tooth socket after an extraction. This globulin substance has been prepared in suitable form for intramuscular injection and shortens the coagulation time. Intramuscular injection is just as efficacious as the intravenous administration.

An extract from human placental tissue has been prepared which, on intramuscular injection, shortens the coagulation time. When given repeatedly, it has protected hemophilic children from hemorrhagic episodes. It is apparently more effective than tissue extracts prepared from other sources.

Patients with hemophilia have been deliberately sensitized to a foreign protein, such as sheep serum, and subsequently given small intradermal injections of the allergen. This may shorten the coagulation time, and the bleeding tendency may be lessened by repeated injections. The value of this form of therapy is questionable as is the advisability of repeated injections of any material during quiescent periods of the disease.

The use of venom from various species of snakes has been advocated but this procedure is of doubtful value. The administration of estrogenic substances and ovarian extracts is of no value in controlling active bleeding or the hemorrhagic tendency.

The pain accompanying hemarthrosis should be controlled by immobilizing the joint by means of molded splints. The administration of salicylates may have some analgesic effect. Gentle massage, heat, and passive motion are indicated after the acute symptoms have subsided. Active use of the joint in the early stages may lead to severe damage and ankylosis.

Although the available methods of treatment leave much to be desired, some of the newer methods, such as placental extract, plasma globulin, and purified thrombin, give promise of great effectiveness in controlling the hemorrhage in hemophilia patients. Intravenous injections of "antihemophilic globulin" result in a marked shortening in the coagulation time of hemophilic blood and it has been found that 200 to 400 mg. of the material will keep the coagulation time at low levels for eight to twelve hours and that repeated injections may be given without the development of a refractory phase. It is to be hoped that further development of this material will result in a substance which can be given in small volume and which may be used prophylactically.

ing is presumably on the basis of capillary weakness without any obvious dilatation of the vessels. There are no alterations in the blood

We have found many patients, particularly females, in whom there is no evidence of telangiectasia and no alterations in the blood but who have a tendency to bruise easily, to bleed excessively from slight trauma, or to have recurring hemorrhages from one source or another. Many of these patients have a positive constrictor test as their only abnormal finding. The hemorrhages may be extremely severe. Such cases have been listed under the unsatisfactory term *tendency to bleed*. Hemorrhage can usually be controlled by one or more small blood transfusions

FIBRINOPENIA

A few cases have been described in which a hemorrhagic tendency was found in association with an absence or lowering of the fibrinogen in the blood plasma. The fibrinogen level at which hemorrhage occurs has not been firmly established and even though the fibrinogen level is continuously low the bleeding may be intermittent. The normal plasma level ranges from 0.2 to 0.4 Gm. per 100 cc.

Fibrinopenia is found more frequently in children as a congenital and usually a familial disease with the hemorrhagic tendency becoming apparent after trauma. The condition may also occur in an acquired form in association with a wide variety of diseases.

VITAMIN K AND ITS RELATION TO HEMORRHAGE

The recognition, synthesis, and clinical application of vitamin K form one of the interesting chapters in the story of investigative medicine. Although the picture is not complete, there has been great progress in our knowledge of this phase of blood coagulation. Dam first observed a hemorrhagic tendency developing in chicks that had been kept on a specifically restricted diet. Soon after this McFarlane and also Holst and Halbrook noted a similar tendency. A diet which prevented this hemorrhagic disease was found to contain a substance which had the characteristics of a vitamin. It was called vitamin K by Dam. The coagulation time of the blood was prolonged during the hemorrhagic state, and the delayed coagulation was found to be due to a deficiency in the prothrombin of the blood plasma. The hemorrhagic tendency could be stopped, the coagulation time reduced

trunk or on the mucous membranes of the nose, mouth, and tongue. Larger lesions, spider angiomas and nodular vascular tumors, may also occur. When venules are involved, the lesions are darker and more purplish in color than those composed of dilated capillaries. The lesions are occasionally encountered in children but ordinarily do not become numerous or large until early adult life. Rupture and hemorrhages are more common with the larger lesions in later life. There have been numerous examples of families through which this trait has been transmitted so that its familial incidence is firmly established.

The most common locations for the lesions and hemorrhages are the skin *of the face and the mucous membranes of the nose and buccal cavities*. They may be present, however, on any mucous membrane, and hemorrhage may occur from any involved area. Hemoptysis, hematuria, melena, or uterine hemorrhage may result. Spontaneous hemorrhage may occur at any time from any of the lesions, and although hemorrhage may precede the recognition of the vascular lesion, it does not occur in the absence of such a lesion. The bleeding may be slight or severe and fatal. Hemorrhages usually become more frequent with advancing age. Hemorrhages from the skin are ordinarily not as severe as those from lesions in the mucous membranes.

Three members of one family observed in this hospital had had repeated episodes of hemorrhage from the base of the left index finger. This had been the only source of bleeding in 2 of these patients whereas the third had bled from multiple areas.

There are no alterations in the blood except for anemia resulting from the loss of blood.

The only treatment which is of value for telangiectasia is cauterization or ligation of the local lesion. Unfortunately, the lesions are frequently inaccessible, and treatment is impossible. The outlook is not good. Rutin in doses of 40 mg. three times a day has been reported to be of benefit in these cases but the value of the drug in this and other conditions has not been fully substantiated.

RECURRENT HEMORRHAGE

In addition to the familial type of telangiectasia there is a form in which similar vascular lesions occur without a familial trait. These produce recurrent epistaxis or hemorrhages from other regions.

A familial tendency to epistaxis and other forms of hemorrhage which is not associated with telangiectasia is occasionally encountered. The bleed-

eliminated by the use of water-soluble preparations of vitamin K and by their parenteral administration.

A low prothrombin level of the blood, which can be corrected by the administration of vitamin K, occurs under the following conditions according to Snell and Butt:

1. After ingestion of a diet which is inadequate in vitamin K.
2. In newborn infants, as the "hemorrhagic disease of the newborn" or "melena neonatorum."
3. From inadequate absorption of vitamin K in the intestinal tract. This may result from a lack of bile due to (1) inadequate secretion, (2) loss of bile through a biliary fistula, or (3) obstruction of the bile ducts from any cause whatsoever. It may also result from inadequate absorption of the vitamin because of various intestinal lesions such as short-circuiting surgical procedures, intestinal obstruction, or diarrheal diseases.
4. Liver disease The liver is directly concerned with the production of prothrombin, and when this organ is damaged, there is inadequate prothrombin formation. In many instances, however, when this organ is the seat of extensive damage, the amount of prothrombin in the blood does not increase in spite of the administration of large amounts of vitamin K.

It appears, therefore, that the factors necessary to prevent hypoprothrombinemia are (1) the presence of bile in the intestinal tract, (2) a diet containing adequate amounts of the vitamin, (3) a normal absorptive surface in the small intestine, and (4) a liver capable of synthesizing prothrombin.

Concentrates of the extract of alfalfa are effective in raising the prothrombin level of the blood when administered orally but they cannot be given by injection. Synthetic derivatives of quinone are easily prepared and inexpensive, and the dosage and strength of the preparations can be easily adjusted. They can be administered by any route and consequently have largely replaced the crude preparations. The most active preparation is 2-methyl-1, 4 naphthoquinone. This may be given by mouth with bile salts or intramuscularly in solution in oil. It is insoluble in water. The dose by the oral route is 2 mg. daily for prophylactic measures or 5 mg. daily when hemorrhagic manifestations are present. From 2 to 4 mg. may be given intramuscularly and will be effective for several days. Various water-soluble quinone derivatives have been tested and found to be effective. These can be given orally without the addition of bile salts and may be administered intravenously. For these reasons they may entirely replace the crude concentrates and the oil-soluble preparations. The water-soluble compound, 4-amino-2-methyl-1-naphthol, may be given to newborn infants in 1 mg. doses either

to normal, and the prothrombin level of the blood plasma returned to its usual level by giving an adequate diet or by the administration of an ether extract of alfalfa which contained the fat-soluble vitamin K.

Subsequent investigations on the vitamin K content of various foodstuffs have shown that its principal source is in the photosynthetic portion of plants and in vegetable oils. It is found in the green leaves of alfalfa, cabbage, spinach, and cauliflower and to a lesser extent in fruits, vegetables, and berries. Animal products are a relatively poor source although some is present in putrefied protein, as in fish meal.

The chief source of vitamin K used in the early experiments was an ether extract of alfalfa although an extract of fish meal also furnished a potent substance. Improvements in the technic of extraction resulted in products of increasing potency and greater purity, and, although the active principle was not crystallized from these products, a substance was obtained which had the properties of a quinone. An entirely separate investigation had previously isolated a substance, phthiocol, from the capsules of tubercule bacilli, and this had been synthesized. Since it was a naphtha quinone, it was empirically tested in the hemorrhagic disease of chicks and was found to be highly effective in its antihemorrhagic properties. The relationship between the quinones and vitamin K was thereby firmly established. Subsequent investigations have studied the effects of various naphtha quinone compounds in an attempt to find the member of the group which is most valuable in combating this type of hemorrhagic disease.

Vitamin K in itself has no coagulant action but is directly concerned with the maintenance of a normal prothrombin level in the blood. The injection of vitamin K into animals with a low prothrombin level produces an increase in the prothrombin of the blood after a period of a few hours. The experimental work of Smith, Warner, and their associates has shown that the liver is directly concerned with the production of prothrombin although this organ may not be the only one involved in its production. Their work indicates that an adequate amount of available vitamin K is necessary for the synthesis of prothrombin. Vitamin K is a fat-soluble vitamin. Its absorption from the intestinal tract is hindered by an absence of bile. Quick suggested that this accounted for the low prothrombin level and the hemorrhagic tendencies which are observed in patients with obstructive jaundice. In the early clinical investigations vitamin K, in the form of an ether extract of alfalfa, was administered by mouth in association with bile salts. These aided in its absorption from the intestinal tract. The necessity of giving bile salts was

Hemorrhagic Disease of the Newborn

Hemorrhagic disease of the newborn is a condition characterized by spontaneous hemorrhages which may occur from any part of the body. It occurs only in newborn infants, usually before the fourth day of life, and never after the tenth day. It is found in about 1 per cent of newborn infants.

This disease is due to a low prothrombin content of the blood plasma and a resultant defect in the coagulability of the blood. Brinkhous and his co-workers have shown that the prothrombin level of all newborn infants is low, being less than half the normal level of adults, and that a further drop occurs from the second to the sixth day of life. Following this there is a slow increase so that the adult level is reached at the end of the first year. The fall in the prothrombin level during the first week of life is of significance because it is during this period that the evidences of hemorrhagic disease usually make their appearance. Even though their prothrombin level is low, most infants do not bleed because prothrombin is converted to thrombin so rapidly in infants that it compensates for the low prothrombin level. There is also some evidence to suggest that an excess of thromboplastin in the blood of infants may be a compensatory factor. Whatever the reason may be, it is obvious that infants do not bleed with a prothrombin level which would be definitely in the danger zone for adults. There is an unusually low prothrombin level in hemorrhagic disease of the newborn. It has been found to be as low as 5 per cent of the normal adult level.

Symptoms

Spontaneous bleeding from any tissue or organ of the body is the characteristic feature of this disease. Purpura does not occur, but ecchymoses and large subcutaneous hemorrhages are common and may be the only evidence of bleeding. Bleeding frequently occurs from the umbilical stump and may be very profuse. Profuse hemorrhages into the intestinal tract are so common that the name "melena neonatorum" has been given to the disease. If instruments were used at the time of delivery, hematomas are found at the site of their application, and severe bleeding may occur from any abrasions of the skin which were produced. Cerebral hemorrhages are common and produce various neurologic manifestations or they may be fatal. Bleeding

intramuscularly or intravenously and repeated at six hour intervals. From 3 to 5 mg. per day may be given with safety to adults. Menadione, U. S. P. is 2-methyl-naphthoquinone which is soluble in vegetable oils but insoluble in water and is administered by mouth in 1 mg doses

Patients with extensive liver disease and liver insufficiency do not respond to either the oral or the intravenous administration of vitamin K. Their prothrombin level cannot be increased by any of these preparations

Hemorrhage Associated with Jaundice

The tendency of the jaundiced patient to bleed has been a matter of grave concern to the surgeon. It has been a prominent cause for the high mortality that accompanied surgical treatment of jaundice, accounting for about 50 per cent of the postoperative mortality. This hemorrhagic tendency has previously been blamed on alterations in the blood calcium, bilirubin, platelets, fibrinogen, and thromboplastin, but the recent investigations have definitely shown it to be due to a low prothrombin concentration in the blood. It has further been shown that an absence of bile in the intestinal tract interferes with the absorption of the fat-soluble vitamin K resulting in a lowered prothrombin production and hypoprothrombinemia. In obstructive jaundice there is an absence of bile in the intestinal tract, and a similar condition exists with a biliary fistula

The bleeding tendency in the jaundiced patient seldom appears spontaneously prior to operation but becomes apparent during the first ten days of the postoperative period, usually from the second to the sixth day. Estimations of the prothrombin concentration in the blood of patients with obstructive jaundice show that the prothrombin level is reduced in a large number of the cases. Hemorrhage is apt to occur when it is below 70 per cent as determined by Quick's method or 60 per cent by Smith's method. A postoperative hemorrhage may occur in a patient whose preoperative prothrombin concentration was above the critical level. This level is reduced by tissue fluids which dilute the blood in restoring a normal blood volume postoperatively.

The preoperative treatment of a jaundiced patient should include the administration of vitamin K in sufficient amounts to bring the prothrombin concentration to its normal level. Vitamin K should continue to be taken during the postoperative convalescent period. Methods for the determination of prothrombin are simple and quickly performed so that the blood level can be followed continuously to govern therapy.

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Examination of the blood reveals a prolonged coagulation time although the bleeding time is normal. The platelets are present in normal numbers, and the clot retracts normally.

Diagnosis

The diagnosis is based on the appearance of the bleeding tendency during the first few days of life and on a low prothrombin level in the plasma. Although the usual types of hemorrhagic disease must be considered in the differential diagnosis of this condition, they seldom make their appearance in a newborn infant. The mortality in untreated cases of this disease is about 50 per cent, but rapid and permanent recovery takes place with proper treatment.

Treatment

The most effective therapy consists in the administration of a water-soluble preparation of vitamin K. This may be given either intramuscularly or intravenously, but the latter route gives the most prompt results. The use of 1 mg. of 4-amino-2-methyl-1-naphthol every six hours until the bleeding ceases has been recommended.

The administration of 1 mg. of this preparation soon after birth has been suggested as a prophylactic measure but does not seem to be a justifiable procedure to follow in all newborn infants. The prothrombin level of the infant can be raised by giving the vitamin to the mother before delivery. This prophylactic administration of vitamin K to those mothers with a low blood prothrombin level or to those in whom a deficiency of vitamin K is suspected may prevent the occurrence of hemorrhagic tendencies in the infant.

Hemorrhage of the newborn may also be controlled by blood transfusion. This supplies directly the prothrombin which is lacking in the blood of the infant. Repeated small transfusions are preferable to a single large one. Blood which has been stored in a blood bank should not be used, it has been shown that the prothrombin content of the blood decreases rapidly during storage.

DICOUMARIN AND THE INDUCED HEMORRHAGIC STATE

Heparin has been used as an anticoagulant in an attempt to lessen the incidence of postoperative venous thrombosis and to prevent the extension of thrombi which have already formed. Heparin is expensive and has not been too satisfactory although it does prolong the coagulation time of the blood.

Its action is immediate but of short duration so that it must be administered intravenously by continuous drip or by repeated injections of 50 mg. doses at intervals of three to four hours.

Dicoumarin has recently been used for the same purpose and possesses certain advantages over heparin. It is inexpensive, nontoxic except for its effect on blood coagulation, and may be given by mouth. Dicoumarin (3,3'-methylenebis-4-hydroxycoumarin) is the active principle found in improperly cured sweet clover hay which produces a hemorrhagic disease in cattle. The substance was isolated from sweet clover and later synthesized by Link and his associates. Dicoumarin delays coagulation by inhibiting the formation of, or by destroying the already formed, prothrombin. It does not damage the liver. The prothrombin time (Quick or Smith method) is prolonged, there is an increased rate of sedimentation of the erythrocytes, and the retraction of the blood clot is inhibited. Too large amounts of the substance cause a prolonged coagulation time, and serious or fatal hemorrhage may occur.

Dicoumarin is administered by mouth in an average dosage of 150 to 300 mg. per day. The dosage must be individualized and regulated by the prothrombin time which should be determined at daily or twice daily intervals. The prothrombin level should be maintained between 20 and 30 per cent. It has been shown that intravascular thrombosis rarely occurs when the percentage of prothrombin is less than 30 and bleeding rarely occurs when the percentage is 10 or more. There is a latent period of about twenty-four hours before its effect becomes evident so that heparin is frequently given for immediate action. Both heparin and dicoumarin are given during the first twenty-four hours so that the immediate action of heparin can be utilized. As the effect of dicoumarin becomes manifest after twenty-four hours, heparin may then be discontinued and only dicoumarin administered. Fifty milligrams of heparin may be given intravenously every four hours or by continuous intravenous drip until the dicoumarin has taken effect. Three hundred milligrams of dicoumarin are given orally with the first injection of heparin and 200 mg. the following day. The subsequent daily dose depends upon the prothrombin level.

The action of dicoumarin may be stopped immediately by transfusions of fresh blood and in most instances synthetic vitamin K (64 mg. of menadione bisulfite) given intravenously will control the bleeding. Dicoumarin is a dangerous drug because of its liability to cause fatal hemorrhage and should be given only when its effect can be followed by determinations of the prothrombin level in the blood.

Anticoagulants are coming into more and more common use in the following conditions: (1) acute coronary occlusion; (2) after nonfatal pulmonary embolism; (3) for thrombophlebitis and phlebothrombosis; (4) for sudden arterial occlusion; (5) as prophylaxis against postoperative venous thrombosis. It should not be used in: (1) ascorbic acid or vitamin K deficiency; (2) renal insufficiency; (3) blood dyscrasias with a bleeding tendency; (4) recent surgical operations, (5) ulcerative lesions or open wounds; and (6) subacute bacterial endocarditis.

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LEUKEMIA

LEUKEMIA IS A SYSTEMIC DISEASE OF UNKNOWN CAUSE CHARACTERIZED BY A rapid and abnormal proliferation of leukocytes in the hematopoietic organs and by the presence of immature leukocytes in the peripheral blood stream. The total leukocyte count is elevated in most of the patients, but the presence of immature cells in the blood stream is a more significant feature than is a rise in the total leukocyte count. The disease is invariably fatal.

Leukemia was first described as a clinical syndrome in 1845 by Bennett and Virchow, whose independent observations were published almost simultaneously. It was called leukocythemia by Bennett and leukemia by Virchow. The following year, 1846, the first ante mortem diagnosis of the disease was made by Fuller. Virchow later recognized that one type of leukemia was characterized by enlargement of the lymph nodes and another presented a markedly enlarged spleen. Neumann studied the bone marrow from patients with leukemia and described a third type, myelogenous leukemia. The staining methods devised by Ehrlich in 1891 showed that the splenic and myelogenous forms were identical. Naegeli in 1900 described the myeloblast and emphasized its relationship to leukemia. Monocytic leukemia was first described by Reschad and Schilling-Torgau in 1913.

Leukemia is most satisfactorily classified according to the type of leukocyte that is predominantly affected. The myelogenous, lymphocytic, and monocytic types of leukemia are the most common. There are rare cases of plasma cell leukemia and megakaryocytic leukemia. From a clinical standpoint all types occur in an acute and a chronic form so that each variety may be subdivided in this manner.

- | | |
|------------------------|---------------------------|
| 1 Myelogenous leukemia | 3 Monocytic leukemia |
| Acute and chronic | Acute and chronic |
| 2 Lymphocytic leukemia | 4 Plasma cell leukemia |
| Acute and chronic | 5 Megakaryocytic leukemia |

The leukemias are further subdivided into leukemic, subleukemic, and aleukemic types or phases depending upon the changes in the peripheral blood. In the leukemic type there is elevation of the total leukocyte count in

the peripheral blood stream and immature cells of the involved series are present. The subleukemic type or phase shows no elevation in the total leukocyte count or a lower than normal count, with immature cells of the involved series apparent in the peripheral blood. The dividing line between the leukemic and subleukemic phase is usually taken arbitrarily as 15,000, a patient showing immature cells but with a leukocyte count below 15,000 is in a subleukemic phase whereas one with a count above 15,000 is in a leukemic phase. The term "aleukemic" leukemia is admittedly poor but by common usage has come to denote those leukemias in which the total leukocyte count is normal or low and in which no immature cells are apparent. It is probable that in all such cases immature cells could ultimately be found after prolonged searching but they are not encountered in the usual careful differential count.

Histologic examination of tissue reveals that the pathologic changes are the same regardless of the number of leukocytes in the peripheral blood so that one cannot differentiate the leukemic, subleukemic, or aleukemic phases by this means. The leukocyte count in the peripheral blood may change at any time from a leukemic to an aleukemic phase or vice versa with no alteration in the clinical manifestations and the height of the leukocyte count is not of definite prognostic value. Likewise the total leukocyte count cannot be used as an absolute indication or contraindication for therapy.

Etiology

The cause of leukemia is unknown, but the two most prevalent theories hold that it is either a neoplastic disease or an infectious process. There are some who look upon it as a deficiency disease in the sense that pernicious anemia is a deficiency state. The neoplastic theory is most widely accepted.

Leukemia consists of a rapid and uncontrolled growth of a particular type of blood cell with a deposition of these cells in various tissues of the body and in some diseases, such as chloroma, the formation of tumor-like masses. There has been a tendency for pathologists to include lymphocytic leukemia in that group of diseases which are characterized by a progressive enlargement of all the fixed lymphatic tissues, the lymphomas or lymphoblastomas, since it presents a similar pathologic picture. The course of leukemia, with its cachexia and invariably fatal outcome, is extremely suggestive of a neoplasm as is the histologic picture. But even if this view is accepted, it does not explain the basic etiology.

The onset and course of the acute leukemias have many of the characteristics of an acute infectious process. Many different organisms have been

cultured from the blood and from the tissues of patients with leukemia. It is difficult to say, however, that these are not secondary invaders which gain a foothold because of a lowered resistance of the host. A leukemia-like (leukemoid) reaction occasionally occurs as a response to an infection, and it is sometimes hard to differentiate such a reaction from true leukemia inasmuch as acute leukemia frequently has an infection of some type as its first recognizable feature. No single organism has been definitely proved to be responsible for leukemia, nor has the disease been transmitted by any strain of organisms. Definite proof of infectious origin is lacking.

Trauma, particularly to bone, has been followed by leukemia. Lewsen found 60 recorded cases in which this relationship existed. In practically all of these the leukemia was of the myelogenous type. Absolute proof that trauma was the causative factor is lacking.

Exposure to certain drugs and chemicals has been followed by leukemia in a few instances. Benzol has been incriminated most frequently, and studies have shown that exposure to this chemical not infrequently causes hyperplasia of the bone marrow with the appearance of immature myeloid cells in the blood stream. Other substances such as pyridine, aniline dyes, and neoarsphenamine have also been blamed. Experimental studies on animals have tended to substantiate the belief that they may produce leukemia or a leukemia-like disease. Exposure to roentgen rays or radioactive substances has likewise been followed by leukemia, and Forkner has summarized these cases. Since radioactive substances are known to affect the bone marrow and also to cause carcinoma of the skin, he believes that irradiation may be a factor in the production of leukemia in some cases although perhaps only in individuals who already have a predisposition to the disease.

Although attempts to transmit leukemia from one patient to another human subject have failed, the disease has apparently been successfully transmitted from humans to experimental animals by the injection of an extract of the urine from a patient with leukemia. Myeloid hyperplasia resulted from the administration of an extract from the urine of patients with myeloid leukemia and a lymphocytic reaction with that from patients with lymphocytic leukemia. It can be transmitted in mice, guinea pigs, and fowls, but the significance of these experimental studies is still in doubt. There is some question as to whether or not the leukemia which is found in man is the same disease as that which occurs in animals and fowls.

Leukemia is widespread throughout the world. It occurs in all races and in all countries. It appears to be more common in the white race than among Negroes and is more frequent in males than in females. There have been a

few instances in which it developed in siblings, in twins, and in a mother and her son. Leukemia and malignant growths have occasionally appeared in the same family. From the available data no definite conclusions can be drawn as to the hereditary nature or the familial tendencies of leukemia. An extremely few cases of congenital leukemia have been recorded.

The incidence of leukemia is rather difficult to determine with even a moderate degree of accuracy. At the University Hospital we encountered 207 cases among 36,420 admissions to the medical and pediatric services, an incidence of 1 case of leukemia for each 175 admissions. Ikeda found 77 cases of leukemia among 12,396 necropsies at the University of Minnesota Hospital. In Denmark the death rate for leukemia was 1 per 50,000 population, statistics for the United States show 2.8 deaths from leukemia per 100,000 population in 1936. A recent study has shown a steady increase in the death

rate from leukemia since 1900. In 1940 there were 3.7 deaths from leukemia per 100,000 population and each year since 1940 there have been over 5,000 deaths from leukemia in the United States. In view of this apparent increasing incidence Dameshek has suggested increased exposure to possible carcinogenic agents as the cause. Ulrich showed statistically that the incidence of leukemia among radiologists is far higher than among other physicians.

The age incidence varies with the type of leukemia (Fig 49). Forkner has stressed the high incidence of acute leukemia in childhood, of chronic lymphocytic leukemia during the fifth and sixth decades, and of chronic myelogenous leukemia during the third and fourth decades of life. The frequency of the various types of leukemia has varied with different observers. In our experience lymphocytic leukemia has been the most common, but

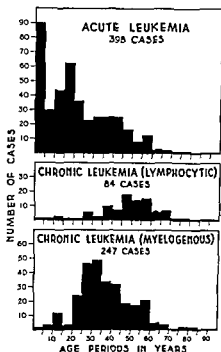


FIG 49. The age incidence of leukemia arranged in five year periods (Redrawn from the data of G Ward, *Brit. J. Child Dis.*)

Osgood reports their frequency as follows: myelogenous leukemia, 62.2 per cent, lymphocytic leukemia, 32.7 per cent, and monocytic leukemia, 5.1 per cent. This relative incidence is approximately the same as the frequency with which the corresponding type of cell appears in normal blood.

ACUTE LYMPHOCYTIC LEUKEMIA

Acute lymphocytic leukemia is primarily a disease of childhood. Its incidence is relatively low in adult life. Although it is reported to have been present at birth in a few instances, such congenital cases are exceedingly rare.



FIG. 50. The age incidence in 61 cases of acute lymphocytic leukemia (Falkenstein and Fowler, *Am. J. Dis. Child.*)

It occasionally occurs during the first year of life but is more frequent during the succeeding five years. In a group of 61 patients with this disease we encountered only 3 in whom the symptoms appeared during the first year of life, their ages were 3½, 5, and 11 months respectively (Fig. 50). The

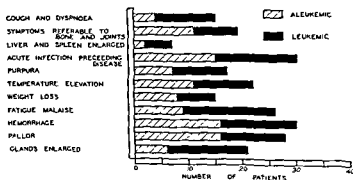


FIG. 51. The frequency with which various entrance complaints were encountered in acute lymphocytic leukemia (Falkenstein and Fowler, *Am. J. Dis. Child.*)

disease is more frequent in males than in females by a ratio of about 2 to 1.

The onset may be abrupt or insidious, but the course is rapidly progressive and the disease is usually of only a few months' duration. The most common

few instances in which it developed in siblings, in twins, and in a mother and her son. Leukemia and malignant growths have occasionally appeared in the same family. From the available data no definite conclusions can be drawn as to the hereditary nature or the familial tendencies of leukemia. An extremely few cases of congenital leukemia have been recorded.

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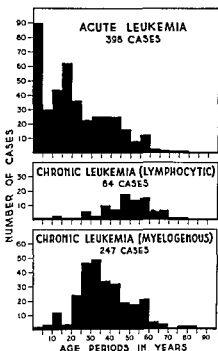


FIG. 49 The age incidence of leukemia arranged in five year periods (Redrawn from the data of G Ward, *Brit J Child Dis*)

Osgood reports their frequency as follows: myelogenous leukemia, 62.2 per cent, lymphocytic leukemia, 32.7 per cent, and monocytic leukemia, 5.1 per cent. This relative incidence is approximately the same as the frequency with which the corresponding type of cell appears in normal blood.

sion of lung tissue by the leukemic cells in 5 of our 61 patients. In those patients with mediastinal obstruction it must be remembered that irradiation therapy first causes some swelling of the lesion under treatment. Consequently a marked and sometimes dangerous aggravation of obstructive symptoms may precede any evidences of improvement.

Pain in the bones and joints is often encountered in acute lymphocytic leukemia in children. This symptom was present in one-third of our patients; in a few instances it was the first symptom of the disease. The clinical picture is suggestive of acute rheumatic fever when joint pains occur early in the course of the disease and are associated with fever, redness and swelling of the joints, a rapid pulse, and pallor. The resemblance is especially confusing if the leukemia is of the aleukemic type. An irregular mottling and osteoporosis of the bones may be detected on roentgenologic examination, but there is no correlation between the severity of the joint pains and the extent of the roentgenologic changes in either the bones or the joints.

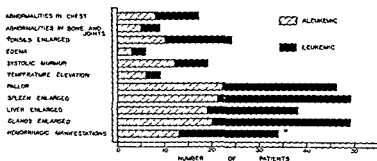


FIG. 52. The frequency with which various abnormalities were encountered on physical examination of patients with acute lymphocytic leukemia. (Falkenstein and Fowler, *Am J Dis Child*.)

Gastrointestinal symptoms are common but usually nonspecific in character. Leukemic infiltration of the intestinal wall occasionally produces severe manifestations, or subserous hemorrhages may cause extreme pain. Hematuria is an infrequent complication. The cardiovascular symptoms are due to the accompanying anemia although an actual leukemic infiltration of the myocardium has been encountered occasionally.

The abnormalities encountered on physical examination are confined mainly to the lymphatic system (Fig. 52). Enlargement of the lymph nodes in acute lymphocytic leukemia is usually less marked than that which occurs in the chronic form of the disease. The nodes are not significantly enlarged in a few instances, but a majority of the patients show a generalized but slight lymphadenopathy. In some instances the lymphadenopathy is extensive

mode of onset is with fatigue, malaise, and listlessness accompanied by pallor and weakness (Fig. 51). In 50 per cent of our patients the leukemia was preceded by an acute infectious process following which the child did not convalesce as rapidly as expected. In other instances the onset is more abrupt and the disease may be ushered in by hemorrhagic manifestations, by chills, fever, and prostration, or by the occurrence of difficult breathing. When acute lymphocytic leukemia occurs in adults, the clinical and hematologic features are similar to those encountered in children although the course may be somewhat less rapid.

The clinical features are variable and nonspecific, but weakness, fatigability, listlessness, and pallor are ultimately encountered in all patients. There may be some loss of weight, usually not extreme, and cachexia is not to be expected. The weakness and fatigability become more marked as the disease advances. Commonly, a continuous or remittent type of fever, frequently reaching 105 F., and associated with marked prostration, suggests the presence of a virulent infectious process.

Hemorrhagic manifestations appear in practically all patients in the terminal stages of the disease. They may also mark its onset. The most common manifestation of the hemorrhagic tendency is purpura. This may occur in the form of small intracutaneous hemorrhages or as larger ecchymotic areas which appear spontaneously or as a result of minor trauma. Epistaxis and hemorrhage from the gums are frequent, and the bleeding may be mild or profuse. Although bleeding from the gastrointestinal tract and from the uterus occur less frequently than does bleeding from the gums and nose, a profuse uterine hemorrhage may be the first symptom of trouble even in young girls prior to the onset of their menses. Ulceration of the mucous membranes of the mouth may occur. The gums may be swollen and spongy from a leukemic infiltration of these tissues. Such an involvement of the gums and buccal cavity is less frequent in lymphocytic leukemia than in either the myelogenous or the monocytic form. Retinal hemorrhages are present in most patients in whom there are any hemorrhagic manifestations, and sudden deafness may result from bleeding into the middle ear. Cerebral hemorrhage is occasionally the cause of death.

Difficult breathing and a distressing cough are extremely troublesome features which occurred in about 25 per cent of our patients. These symptoms are usually due to enlarged mediastinal lymph nodes causing pressure on the trachea. A dry rasping type of cough frequently develops. Edema of the neck and upper extremities with engorgement of the superficial veins may result from mediastinal obstruction. Pleural effusion may account for shortness of breath in other instances, but there was actual infiltration and inva-

enopathy tends to be somewhat less marked. The difficulties in diagnosis are greater; the condition must always be borne in mind in patients with a severe progressive anemia which is otherwise unexplained. It is usually accompanied by thrombopenia, and the smear may show a relative lymphocytosis. It can be differentiated from aplastic anemia by the lymphadenopathy, splenomegaly, and by sternal puncture.

Anemia is present in all patients with acute lymphocytic leukemia. It may be mild in the early stages but progresses steadily and rapidly to become one of the most prominent features. The red cells appear normal or slightly

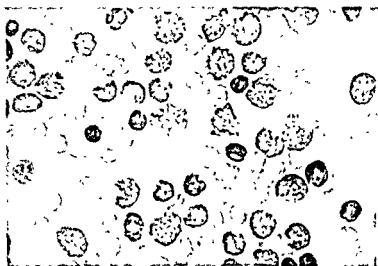


FIG. 53 Photomicrograph of a blood smear in acute lymphocytic leukemia. A few small mature lymphocytes are evident, but most of the cells are large immature forms.

hypochromic, and the color index remains about normal. The usual symptoms of anemia will be present and may be the main feature of the illness. Nucleated erythrocytes in varying numbers are often encountered.

The platelets are diminished in number, frequently in the early stages of the disease. Accompanying the thrombopenia is a prolonged bleeding time, a nonretractile clot, and a positive constrictor or arm band test. This secondary thrombopenic purpura accounts for the hemorrhagic symptoms that are so common and so dangerous.

The basal metabolic rate is elevated to a degree roughly proportional to the severity of the course. Bence Jones protein may be present in the urine, which also contains albumin and casts. The blood uric acid is usually increased in this and other leukemias. Blood cholesterol may be lowered.

The mediastinal nodes are particularly apt to become much enlarged in children. The spleen is usually palpable, but an extreme degree of splenomegaly is rare. The liver is slightly enlarged in many cases.

Infiltration of the skin is not as common in the acute as in the chronic form of the disease. When encountered it is frequently in the form of raised nodular areas of varying size and number. These skin infiltrates have appeared before there are changes in the peripheral blood although the bone marrow shows the typical infiltration. Some cases with this type of involvement have pursued a very rapid course after the blood changes have become apparent.

Blood

Acute lymphocytic leukemia may occur in a leukemic or an aleukemic phase. The leukocyte count may shift from one phase to the other. In the leukemic stage the leukocyte count is elevated, and 1,000,000 or more cells per cubic millimeter of blood are occasionally encountered. The count is usually lower than this and as a rule does not become as high in the acute as in the chronic form of the disease. The predominant cell on the blood smear is the lymphoblast, a large cell with a round or oval nucleus containing one or more nucleoli. The chromatin forms a fine net work which is evenly distributed throughout the nucleus. The cytoplasm is basophilic and contains no granules. Degenerated cells or "basket cells," which consist of only a smeared-out nucleus, are frequently noted. They may comprise a majority of the leukocytic elements on the blood smear. A few small mature lymphocytes may be seen (Fig. 53). Cells in all stages of development between the lymphoblast and the mature lymphocyte will be found on the smear and only an occasional neutrophil. Usually, the more acute the course of the disease, the higher will be the percentage of immature cells.

The subleukemic phase of acute lymphocytic leukemia is characterized by a total leukocyte count of 15,000 or less with immature cells in the peripheral blood. The aleukemic phase presents a normal or low leukocyte count with almost no immature cells. Undoubtedly immature cells enter the peripheral blood stream in every case but so rarely that they are undetected in a careful differential count. The leukocyte count may drop to 1,000 or less and this aleukemic phase may persist throughout the entire disease or a leukemic phase may supervene at any time. Likewise a leukemic state may become subleukemic or aleukemic. The aleukemic form is far more common than was formerly supposed.

The clinical course of those cases which are permanently aleukemic is not significantly different from that of the leukemic type although the lymphad-

ment of the lymph nodes, splenomegaly, or immature cells in the blood stream, nor are nucleated erythrocytes encountered. A biopsy of the sternum or of a lymph node may be necessary for a final interpretation.

Thrombopenic purpura is a part of the picture of acute leukemia. This secondary form of the disorder must be differentiated from the idiopathic variety. The presence of immature lymphocytes, the progressive anemia, and lymphadenopathy found with leukemia serve for differentiation.

Acute exacerbations are of common occurrence in the course of chronic lymphocytic leukemia. During these episodes the clinical and hematologic pictures are similar to those of acute leukemia.

Course

Acute lymphocytic leukemia is invariably fatal, usually within six months. The average duration of life in the 61 cases from this clinic was eighteen weeks. One patient died a week after the onset of symptoms; one after fifty-six weeks. Death may be due to the progressive weakness and anemia or hemorrhage, intercurrent infection, or mediastinal obstruction.

Treatment

Treatment is unsatisfactory. Deep roentgen ray therapy may relieve obstructive symptoms in the mediastinum or relieve the pain in the bones and joints; otherwise it is of no value. Such therapy makes the condition worse in many instances by markedly increasing an already elevated basal metabolic rate. Roentgen ray therapy should not be used except to relieve specific pain or obstructive symptoms.

Transfusions give temporary symptomatic improvement but are of only transient benefit.

Aminopterin, a folic acid antagonist, has been tried but is extremely toxic and still purely in an experimental stage. The results have been encouraging, with temporary remissions in some patients. It is not curative.

CHRONIC LYMPHOCYTIC LEUKEMIA

Chronic lymphocytic leukemia is primarily a disease of middle and late adult life. In contrast to the acute form it is encountered only rarely in children. It is characterized by generalized lymphadenopathy and hyperplasia of all the lymphatic tissues of the body and, in the blood stream, by a lymphocytosis and varying numbers of immature lymphocytes.

Pathologically the disorder is characterized by lymphoid hyperplasia and infiltration so that the architecture of the lymph nodes and lymph nodules is replaced to varying degrees by masses of small or medium-sized lympho-

Sternal puncture reveals the marrow to be crowded with cells of the lymphocytic series which have replaced the normal myeloid tissue. The percentage of cells of the erythrocytic and myeloid series is far below normal. The lymphocytes are found in varying stages of immaturity with the lymphoblast predominating. Infiltration of the marrow has been found before there are significant changes in the peripheral blood and we have encountered one child in whom the characteristic bone marrow changes were discovered at a time when the only manifestation was an unexplained fever. The leukemic and aleukemic phases of the disease present the same marrow picture.

Pathology

There is no difference in the histologic picture of the tissues in cases which have a leukemic blood picture and in those in which it is aleukemic. The lymph nodes are enlarged, and the architecture is destroyed by an overgrowth of lymphoid cells which are large and immature. These cells predominate in all the lymphoid tissues throughout the body. Organs and tissues of the body are infiltrated to varying degrees by these lymphoblastic cells, particularly the spleen, liver, and to a lesser extent the bone marrow.

Diagnosis

Acute lymphocytic leukemia must be differentiated from other forms of acute leukemia. This can usually be accomplished by examination of the blood smear. If the cells are so immature as to be unidentifiable, some help may be obtained with the peroxidase stain although this is of little value with extremely immature cells. Sternal puncture or microscopic examination of a node taken for biopsy may be necessary to establish the diagnosis. These procedures may fail to classify the leukemia in some instances in which a vast majority of the cells are very immature. Such cases are rare but have been diagnosed simply as acute "stem-cell" or "blast cell" leukemia.

Infection may produce a leukemoid blood picture of the lymphocytic type, but seldom shows the progressive anemia, lymphadenopathy, splenomegaly, prostration, and thrombopenia of leukemia.

Infectious mononucleosis during its acute phase closely simulates acute lymphocytic leukemia. The characteristic cells of the former disease are large abnormal lymphocytes rather than immature cells. There is no significant degree of anemia or thrombopenia. The heterophile antibody test is an aid in the diagnosis of infectious mononucleosis.

Aplastic anemia is closely simulated by the aleukemic form of acute lymphocytic leukemia as it presents anemia, thrombopenia, and leukopenia with a relative lymphocytosis. Aplastic anemia does not produce enlarge-

a widened mediastinum is detected on physical or roentgenologic examination, and pressure symptoms may occur from encroachment on the trachea or on the vessels and nerves in this region. Histologic evidence of involvement of the mediastinal nodes is obtained at necropsy even though there was no clinical or roentgenologic evidence of enlargement. The abdominal and retroperitoneal nodes may become large enough to be palpable as firm irregular masses but more frequently they are not large enough to be detected. In a few instances they are sufficiently enlarged and so situated that they produce symptoms from their pressure on the common bile duct, intestine, ureters, or other structures. Ascites may occur but is not common. Since the lymphadenopathy is generalized, the variety of symptoms from pressure of enlarged nodes on various structures is almost unlimited.

The spleen is always involved and is sufficiently enlarged to become palpable in practically all patients with this disease. Its size is variable. In one patient it may extend a hand's breadth below the costal margin, in another it may be barely palpable. The extreme degree of splenomegaly found in chronic myelogenous leukemia is not commonly encountered in the lymphocytic type. The liver becomes slightly enlarged in most patients but only in rare instances is it extremely large.

Extremely few cases of chronic lymphocytic leukemia have been reported in which there was no demonstrable enlargement of any of the lymph nodes although the blood picture was characteristic of the disease.

Weakness, fatigability, and pallor may be the first symptoms of this disease but they usually appear only after the lymphadenopathy has become apparent. These symptoms are progressive although the rate of their progression varies in different patients and from time to time in the same patient. There may be no symptoms of the disease for many years in the very chronic form, and the only manifestation will be the persistent lymphadenopathy. In other instances there is a slight fatigability which does not progress but remains constant over a long period of time. Such patients may fulfil their life expectancy and die of an unrelated condition. An acute episode which is suggestive of acute leukemia may supervene at any time. This may subside to a chronic state or progress to a fatal termination. In the late stages of the disease there is loss of weight and cachexia in addition to a marked loss of strength. Anemia with its associated symptoms may be present in the advanced stages and in many instances is the predominant manifestation. It is so mild as to cause little or no trouble in the very chronic forms.

cytes. This infiltration is found in any part of the body where there is lymphoid tissue. The spleen, liver, and bone marrow are invariably involved. There may be invasion of organs in which lymphoid tissue is not a conspicuous part of the normal histologic picture. The spleen is enlarged to varying degrees, and the normal architecture is obscured by the overgrowth and infiltration of lymphocytes. Infarcts and perisplenitis are frequently encountered. The bone marrow is similarly infiltrated, often to such an extent that the lymphoid cells overshadow the normal bone marrow cells.

The disease has been classified by some pathologists as the lymphocytic type of malignant lymphoma since the histologic picture is so similar to lymphosarcoma and lymphoblastoma.

Chronic lymphocytic leukemia is most frequently encountered between the ages of 45 and 60 and is extremely rare below the age of 10. It occurs about twice as frequently in males as in females.

Clinical Features

The onset of chronic lymphocytic leukemia is so gradual and insidious that no more than an approximate beginning date can be determined. The accidental discovery of enlarged lymph nodes or a gradually increasing weakness and fatigability are the manifestations which most frequently bring the patient to a physician. Hemorrhagic symptoms or purpura may occasionally be the first recognizable manifestation, but this is unusual. The disease is not infrequently detected on a routine physical examination or on a routine blood count in a patient who complains of some entirely unrelated disorder.

The most characteristic feature is the generalized lymphadenopathy. The enlarged nodes may occur singly, or chains of nodes may be involved with the size of the individual members varying from a few millimeters in diameter to 4 or 5 cm. It is unusual to find extremely large masses of lymph nodes or single nodes such as those encountered in Hodgkin's disease. The nodes are rather firm and rubbery in consistency, not tender, and movable; the overlying skin is not involved. The capsule is not invaded so that the nodes do not become matted together or immobilized. It is usual to find all the superficial nodes involved to about the same degree rather than to have extremely large nodes in one area with slight or no involvement elsewhere. The lymphadenopathy may be generalized at the time the disease is first recognized, or palpable nodes may be found in one or more areas with a gradual extension to other regions. The size of the nodes may vary slightly from time to time. The mediastinal lymph nodes may become enlarged to the extent that

Occasional deafness from leukemic infiltration about the middle ear and vertigo and tinnitus are encountered

Blood

Anemia is a part of the hematologic picture of lymphocytic leukemia, but its severity varies greatly from patient to patient. In the very chronic form of the disease, in which lymphadenopathy is the only clinical manifestation, the anemia is slight or negligible. In the more rapidly progressive forms it becomes more severe. In the acute exacerbations or in the terminal stages of the illness it becomes a very prominent feature. The erythrocytes appear normal or slightly hypochromic, and the color index remains normal or but slightly lowered. The anemia of lymphocytic leukemia is myelophthitic in origin, but in rare cases there may be a hemolytic element with icterus and increased fragility of the erythrocytes. Nucleated erythrocytes are not infrequently found on the smear but are not numerous or constantly present.

The platelets are usually not affected until the late stage of the disease when they are markedly reduced. With this reduction in the number of platelets the other laboratory features of thrombopenic purpura appear (prolonged bleeding time, positive constrictor test, and nonretractile clot), and hemorrhagic symptoms may occur.

Chronic lymphocytic leukemia may appear in a leukemic, subleukemic, or aleukemic form. In the leukemic variety the total leukocyte count is elevated to a variable degree, usually lower than that encountered in myelogenous leukemia. The count may infrequently reach 1,000,000 or more, in our experience between 50,000 and 100,000 is most frequent. The count is subject to wide fluctuations from day to day.

In the very chronic and slowly progressive forms of the disease the predominant cells are the small mature lymphocytes, which comprise from 90 to 95 per cent of all the white blood cells on the smear (Fig. 54). A few larger and more immature cells are encountered but they are not numerous and lymphoblasts are infrequent. In the more rapidly progressive cases or in the acute exacerbations of the disease a higher percentage of the cells in the blood stream will be immature so that some idea of the rapidity of the course can be obtained from examination of the smear alone. Azure granules are less often encountered in the cytoplasm of the young lymphocytes than in mature cells. The blood picture in lymphocytic leukemia is monotonous, all of the cells being similar in their structural characteristics; there are no striking variations in the structure of the lymphocytes as are found in the maturation of the myeloid cells.

A low grade fever, either continuous or remittent in type, is common during the active stages of the disease although it is not as prominent as in Hodgkin's disease. Infiltration of the salivary and lacrimal glands may occur and produce Mikulicz's syndrome, ptosis of the eyelids, and excessive salivation and lacrimation.

The basal metabolic rate is frequently elevated in chronic lymphocytic leukemia, and readings may be +20 to +30 per cent in the active stages. This may be associated with excessive sweating, intolerance to heat, loss of weight, and tachycardia. Such symptoms are less marked in this than in the acute form of the disease, in which the metabolic rate may be much higher.

Purpura and other hemorrhagic manifestations are rare until the late stages of chronic lymphocytic leukemia but ultimately appear in a majority of the cases. The typical hemorrhagic manifestations of thrombopenic purpura are then present. Patients frequently bruise easily even in the early stage of the disease when the platelets are unaltered and small ecchymotic areas are commonly encountered.

Leukemia cutis or infiltration of the skin by leukemic cells may occur. It is far more common in lymphocytic than in myelogenous leukemia. There may be either a diffuse and generalized skin involvement with thickening, dryness, and itching or circumscribed nodular lesions. The nodules vary in size from a few millimeters to 2 or 3 cm. in diameter, and their color varies from yellowish brown to red or purple. There may be vesicles, papules, pustules, or nodules in which specific leukemic infiltrations are found.

Since the gastrointestinal tract is rich in lymphatic tissue which becomes involved in the general leukemic reaction, it is not surprising to encounter many symptoms referable to this tract. They may be vague and nonspecific complaints of anorexia, nausea, vomiting, dyspepsia, flatulence, and diarrhea, or there may be severe pain and obstructive symptoms due to actual invasion and infiltration of the intestinal wall.

Symptoms referable to the circulatory system are primarily due to the accompanying anemia, but there is occasionally an actual invasion of the myocardium by leukemic cells. Infiltration of the lung tissue is not infrequent and has been found in as high as 30 per cent of a series of autopsied cases.

Pain in the bones and joints may occur in chronic lymphocytic leukemia. The former is more frequent in myelogenous leukemia, the latter in the acute form of lymphocytic leukemia. Neurologic manifestations may appear as a result of leukemic infiltrations in the brain or spinal cord or from the pressure of small tumor-like nodules. Cerebral hemorrhage occasionally complicates the clinical picture. There may be hemorrhages in the ocular fundi as well as engorgement of the veins and leukemic infiltrations of the perivascular tissues.

Occasional deafness from leukemic infiltration about the middle ear and vertigo and tinnitus are encountered.

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Basket cells (degenerated cells, naked nuclei) are frequently found on the smear in lymphocytic leukemia. These structures represent cells which have disintegrated so as to leave only the nucleus, which may be but slightly larger and lighter staining than normal or it may be smeared out in many bizarre forms (Fig. 55). Nucleoli may be very plainly visible in those nuclei which

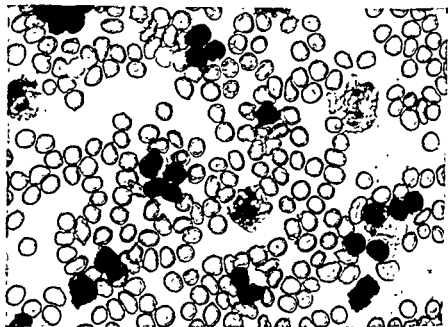


Fig. 54. Photomicrograph of a blood smear of chronic lymphocytic leukemia showing a predominance of small mature lymphocytes with four "basket" or "smudge" cells.

are distorted and spread out. Basket cells are usually immature lymphocytes and are often a prominent feature in lymphocytic leukemia

In the aleukemic type of lymphocytic leukemia the total leukocyte count is normal or low. In some instances there is a high percentage of lymphocytes in the differential count; in others the distribution of cells is found to approach the normal. Few or no immature cells are to be found so that the diagnosis must be based on the histologic changes in the lymph nodes which are the same in the aleukemic as in the leukemic form.

Sternal puncture reveals the marrow to be densely infiltrated by cells of the lymphocytic series, most of which are very immature although more mature lymphocytes are encountered than in acute lymphocytic leukemia. These replace to a large extent the normal myeloid and erythrocytic cells.

Course

The course of chronic lymphocytic leukemia varies greatly from one case to another. As a rule, the later in life the disease makes its appearance, the slower will be its course. The disease may go on for ten, fifteen, or even twenty years after the onset of symptoms and then be only a contributory



FIG 55. Photomicrograph of a blood smear from a patient with lymphocytic leukemia. This shows a small mature lymphocyte, an immature lymphocyte with nucleoli, and a "basket" or "smudge" cell.

cause of death. This exceedingly benign form is not common. The usual duration of life is from three to four years, and the disease is characterized by remissions or latent stages followed by exacerbations of weakness and fatigability with elevation of the leukocyte count and larger numbers of immature cells in the blood stream. The disease is universally fatal.

A sudden increase in the leukocyte count with the appearance of many immature cells in the blood stream is of grave significance. A sudden drop in the count is also serious. The onset of bleeding tendencies adds to the gravity of the prognosis. Patients with lymphocytic leukemia are more susceptible to infections than are normal individuals so that an intercurrent infection is often the terminal event. In some instances, however, an infectious process has apparently initiated a remission in the disease.

Diagnosis

The diagnosis of chronic lymphocytic leukemia is easily made in the presence of the typical blood picture. The greatest difficulty is encountered

in those patients in an aleukemic phase. A lymph node may be removed for histologic examination when the blood picture is not diagnostic. A few conditions, such as infectious mononucleosis or an infection accompanied by an abnormal lymphocytic response, may occasionally simulate the blood picture of lymphocytic leukemia.

Treatment

Irradiation is the most effective form of therapy in chronic lymphocytic leukemia. The subjective improvement which follows irradiation of the spleen and lymph nodes is sometimes striking. The sense of fatigue and weakness disappears. There is a reduction in the basal metabolic rate with relief from the symptoms of hypermetabolism. The decrease in the size of the liver and spleen relieves the patient of the sense of weight and pressure which may have been present from these organs. The leukocyte count can be brought to the desired level by irradiation therapy, but there is no direct correlation between the height of the count and the severity of the symptoms so that a reduction in the count does not in itself give subjective improvement. When large masses of lymph nodes are producing pressure symptoms, roentgen ray therapy will reduce their size and relieve the pressure.

There is no need for irradiation therapy in many of the very chronic cases which are practically asymptomatic.

The response to irradiation is far better in the early stage of the disease, both subjectively and in respect to reduction in the size of the nodes. The response is less marked with each subsequent treatment.

Care must be taken not to reduce the leukocyte count to dangerously low levels as leukopenia may easily be produced. The leukocyte count must be followed closely during the course of therapy and the treatments discontinued before it has reached normal since it continues to fall for a short time after the treatments have been stopped.

The duration of life is probably not much prolonged by irradiation therapy, but it temporarily relieves many of the symptoms and decreases the size of the glands so that pressure symptoms disappear. It may slow the progress of the disease to some extent and convert a subacute course into a more chronic form. The indication for this form of therapy is not so much the height of the leukocyte count as the presence of weakness, fatigability, loss of weight, progressive anemia, or a marked lymphadenopathy.

Irradiation sickness occurs in a large percentage of the patients receiving this form of therapy and is especially severe when it is applied to the liver,

spleen, or other abdominal organ. It may occur, however, with treatment of any area. The predominant manifestations are anorexia, nausea, vomiting, diarrhea, and weakness. The duration of irradiation sickness is short after the treatments are discontinued.

Irradiation therapy should be stopped if the patient's condition becomes worse, especially if loss of weight, profuse perspiration, or progressive anemia develop. It should also be stopped when a rapidly falling leukocyte count approaches normal as there is danger of producing a leukopenia. However, irradiation may be used in a patient who is already in an aleukemic phase of the disease. The higher the leukocyte count at the onset of therapy, the more rapid and greater will be the drop so that in an aleukemic stage there is but a slow and slight drop in the count.

The recent investigations on the use of radioactive phosphorus and other substances with artificially induced radioactivity have given promising results but do not seem to be as effective as roentgen ray therapy. Lymphocytic leukemia does not appear to respond as well to radiophosphorus as either myelogenous leukemia or polycythemia vera although prolonged remissions have been induced. The use of these radioactive substances is still in an experimental stage. The nitrogen mustards have also been used but the results are less satisfactory than in Hodgkin's disease or lymphosarcoma.

Urethane (ethyl phenylcarbamate) has been tried experimentally in the treatment of lymphocytic leukemia and a reduction in the leukocyte count has resulted in some patients but it does not seem to be as satisfactory in this condition as in myelogenous leukemia.

Arsenic in the form of Fowler's solution has been used but is not effective.

Transfusions, of temporary benefit in giving symptomatic relief to those patients with severe anemia, are seldom required in chronic lymphocytic leukemia except terminally. They may temporarily decrease the hemorrhagic manifestations when the platelets are decreased in number.

No dietary restrictions are necessary. The patient's activities need not be restricted except for the limitations imposed by the subjective symptoms.

ACUTE MYELOGENOUS LEUKEMIA

Acute myelogenous leukemia is primarily a disease of young adults although it occasionally occurs in infants and children. We found only 3 cases of acute myelogenous leukemia in children under 16 years of age during the same interval in which there were 61 cases of acute lymphocytic leukemia,

and Wollstein and Bartlett found only 1 case of acute myelogenous leukemia to 14 of the acute lymphocytic type. The disease is likewise rare in late adult life although acute exacerbations of chronic myelogenous leukemia may present a clinical and hematologic picture which is similar to the acute variety. The disease is two to three times as common in males as in females.

Pathology

In myelogenous leukemia the hematopoietic bone marrow, which is normally red in color, becomes gray or reddish gray and has a glistening, gelatinous appearance. The yellow or fatty marrow is encroached upon and replaced to a varying degree by the hyperplastic cellular type of marrow. Myelocytes and myeloblasts predominate in the histologic picture. Not only are the neutrophilic myelocytes increased but the eosinophilic and basophilic forms as well. In some instances the number of megakaryocytes is greatly increased. The erythropoietic elements of the marrow are crowded out by the proliferation of the myeloid cells, and although normoblasts and other immature cells of the erythrocytic series are encountered, they do not form as large a proportion of the cellular elements as is normal.

The spleen is enlarged, the size increasing with the duration of the disease. The most extreme degrees of splenomegaly are found in chronic, long-standing cases rather than in acute forms. The organ is smooth and maintains its normal shape in spite of the increase in size. The consistency varies, being rather soft in the acute cases but becoming more firm as fibrosis develops. The capsule may be thickened and scarred as a result of the *perisplenitis* which follows infarctions. On microscopic examination the normal architecture of the spleen is lost, and the organ densely infiltrated with myeloid cells, myeloblasts predominating in the acute disease and myelocytes or other more mature forms in the more chronic form.

The liver becomes enlarged and shows perivascular infiltration with myeloid cells. The lymph nodes are not enlarged until the late stages of myelogenous leukemia although varying degrees of infiltration with myeloid cells will be found histologically. There are varying degrees of leukemic infiltrations in almost all other organs and tissues of the body.

The presence of myeloid cells in the various organs and tissues has been considered to be an infiltration by cells which have been carried to those locations by the blood stream but which originated in the bone marrow. This "infiltration" is as intense and as generalized in the aleukemic as in the

leukemic type. A more acceptable explanation for the widespread distribution of these cells is that there is a metaplasia of the reticulo-endothelial cells in the various organs, a reversion to their fetal potentialities of producing leukocytes. The reticulo-endothelial cells maintain the potential ability to form myeloid cells so that leukemia may represent a metaplasia of these tissues and a reestablishment of their fetal leukopoietic functions rather than an infiltration of the various tissues by blood-borne cells.

Clinical Features

The onset of acute myelogenous leukemia may be sudden or gradual. A sudden onset is usually characterized by symptoms which are suggestive of a severe infection with fever, chills, profuse perspiration, and possibly sore throat or other localizing manifestations. Even in those with a less abrupt onset there is frequently a history of a preceding infection, commonly of the upper respiratory tract. We have observed several patients in whom the disease developed rapidly after the extraction of a tooth. Hemorrhagic manifestations, either purpura or profuse hemorrhage from some mucous surface, are not infrequently the first symptom. In many instances the bleeding is exceedingly difficult or impossible to control. Ulceration of the buccal mucous membrane accompanied by pain, local swelling, and regional lymphadenopathy may be the presenting symptom.

In other instances the onset is more insidious and is characterized by symptoms of severe and progressive anemia: weakness, pallor, lassitude, shortness of breath, and palpitation. Purpura, painful bones or joints, menstrual disorders, or gastrointestinal symptoms may be the first manifestation. We have encountered 3 cases of acute myelogenous leukemia in which the first symptom was a peculiar numbness of the lower jaw.

The symptoms of acute myelogenous leukemia are similar to those of the acute lymphocytic type, and the course is essentially the same. There is marked prostration and weakness together with the symptoms of a severe and progressive anemia. Hemorrhages from the mucous membranes occur at some time during the course of the illness in most instances. The gums are frequently swollen, edematous, and infiltrated, frequently to the point that the teeth are completely buried (Fig. 56). This is accompanied by bleeding, which begins spontaneously or as a result of slight trauma. Ulcerations of the pharynx and buccal mucosa are common, and a necrotic membrane covers the ulcerated area. The surrounding tissues are edematous and swollen. These

ulcerations may be extremely painful, and the edema of the pharynx may be so extreme as to cause respiratory difficulty. The necrotic tissue and the decaying blood in the mouth give an extremely offensive odor to the breath.

Deafness may occur as a result of hemorrhage into the middle ear or from involvement of the bone. Ménière's syndrome may sometimes be produced. Hemorrhages in the ocular fundi are usually present, and hemorrhages or leukemic infiltrations may occasionally occur in the central nervous system.



FIG 56 Swollen infiltrated gums in a patient with acute myelogenous leukemia

There may be tenderness and pain in the bones, especially in the sternum and ribs, but painful joints are more frequent in acute lymphocytic leukemia than in the myelogenous form. Attacks of abdominal pain may suggest acute appendicitis or intestinal obstruction.

The spleen is enlarged to a moderate degree in acute myelogenous leukemia but does not become as large as in the chronic cases nor is it as firm in consistency. The liver may be slightly enlarged but there is no lymphadenopathy.

Roentgenologic examination may show erosion of the cortex of the bones or fine perpendicular spicules extending outward from the periosteum. Diffuse osteoporosis, localized osteolytic areas, and subperiosteal swelling have been encountered. In some bones there are small areas of destruction surrounded by a sclerotic reaction. Roentgenologic changes in the bones are

prone to occur in younger patients, but considering the extensive medullary involvement, it is surprising that such roentgenologic evidence of bone involvement is not more common

Blood Findings

The total leukocyte count in acute myelogenous leukemia is variable, frequently being around 100,000 or 200,000 but occasionally as high as 1,000,000 or more. The total count is sometimes normal or low, but an aleukemic reaction is rarer in acute myelogenous than in acute lymphocytic leukemia.

In the leukemic form, in which there is an elevated leukocyte count, the predominant cells on the blood smear are the myeloblast and myelocyte. The more acute the disease, the higher will be the percentage of myeloblasts. Variable numbers of the more mature cells of the myeloid series are present, but mature cells with segmented nuclei are not numerous in the acute form. A majority of the myelocytes are of the neutrophilic type although eosinophilic and basophilic myelocytes appear, occasionally to the point of predominating in the differential count. Such cases have been designated as eosinophilic or basophilic leukemia although they are merely a variety of the myelogenous form. The structural characteristics of the myeloblast have been discussed. As was pointed out, they are so similar to those of the lymphoblast that it is difficult to distinguish between the two. There are usually a sufficiently large number of myelocytes and metamyelocytes present to indicate the type of blast cell with which one is dealing.

In the subleukemic phase the total leukocyte count is normal or low, with a smaller percentage of immature cells. Some evidences of immaturity of the cells will always be found. In the aleukemic form only careful search will find the extremely few immature cells in the peripheral blood stream.

A progressive anemia is characteristic of acute leukemia and is usually severe by the time the disease is recognized. It is steadily progressive and becomes severe in the terminal stage. The color index remains about normal but may be slightly lowered.

The platelets are usually decreased in number in acute myelogenous leukemia and may almost completely disappear from the blood stream. The other laboratory features of thrombopenic purpura appear (positive constrictor test, prolonged bleeding time, and a nonretractile clot), and hemorrhagic features develop in the clinical picture.

The phagocytic power of the leukocytes in patients with leukemia is diminished, and the cells are more fragile than normal. In rare instances the

myelocytes or promyelocytes contain small red rod-shaped bodies in their cytoplasm which are termed Auer bodies. These rods may occur singly, or there may be several in the cytoplasm of one cell. They are oxidase positive and are most frequently encountered in very acute cases and in chloroma. The nature of the Auer body is unknown.

Sternal puncture reveals a marrow which is exceedingly hypercellular with a predominance of myeloblasts. The proportion of cells of the erythrocytic series is markedly reduced.

Course

The course of acute myelogenous leukemia is steadily downward, although infrequent remissions may occur. Those patients in whom there is an abrupt onset of the disease are apt to have a very rapid course. The duration of life varies from one week to six months. Warren found that 84 per cent of the patients died within two months of the onset of symptoms. The cause of death is variable. The terminal stage may be characterized by a progressive anemia with weakness and prostration, or death may result from an intercurrent infection or hemorrhage. The disease is always fatal.

Diagnosis

The diagnosis of acute myelogenous leukemia is based primarily upon the blood picture, which typically is sufficiently characteristic to leave little doubt as to the disease. Sometimes, the cells in the peripheral blood cells are so immature that it is difficult to distinguish this type from the lymphocytic or monocytic variety, and many such cases have been diagnosed simply as acute or blast-cell leukemia. Sternal puncture or biopsy should establish the diagnosis.

Aplastic anemia causes more difficulty in differential diagnosis than any nonleukemic disease when the leukemia is in the aleukemic state. In aplastic anemia the spleen is not enlarged, immature leukocytes are not present in the blood stream, and nucleated erythrocytes and reticulocytes are seldom encountered. Aspiration or biopsy of the sternal bone marrow may be necessary to distinguish between the two diseases.

Thrombopenic purpura is usually present in acute myelogenous leukemia and this must be differentiated from the idiopathic form. The anemia of idiopathic thrombopenic purpura is no more severe than can be accounted for by the hemorrhage and is not progressive in the absence of blood loss. A moderate leukocytosis may occur as a result of hemorrhage but the evi-

dences of immaturity of the cells are not marked. Examination of the blood smear usually suffices for differentiation without recourse to sternal puncture.

Agranulocytosis may be suspected in the aleukemic phase of leukemia but it is not accompanied by anemia, thrombopenia, or hemorrhage, and immature cells are not present.

Leukemoid reactions resulting from infections or other conditions may simulate acute myelogenous leukemia. Evidences of the primary disease will be present, however, to serve as a distinguishing point in most instances.

Acute exacerbations of a chronic myelogenous leukemia will present symptoms and findings similar to those of the acute variety, but the history of previous episodes will usually suffice to distinguish the two. The spleen is usually larger in the chronic form than in the acute cases but this characteristic is not distinctive enough to serve as a differential point.

Treatment

There is no form of treatment that is of definite value in acute myelogenous leukemia. Roentgen ray therapy is apt to be more harmful than beneficial because of its tendency to increase the basal metabolic rate, which is already high, and also because of the irradiation sickness which it engrafts upon the constitutional symptoms already present. In rare instances it may give some temporary relief but it is contraindicated in most cases.

Transfusions may decrease the hemorrhagic tendency but seldom stop it entirely. Although a transient subjective improvement in those symptoms which are due to the anemia may be noted after a transfusion, the effects are of short duration. Transfusions are of little use.

Aminopterin and other folic acid antagonists may produce a temporary remission with both the marrow and the peripheral blood returning to near normal. The results are transient. The drugs are extremely toxic and still in an experimental stage.

CHRONIC MYELOGENOUS LEUKEMIA

Chronic myelogenous leukemia is a disease of middle adult life with the peak of its incidence occurring during the third and fourth decades. It is rarely encountered in children and is infrequent in very late adult life. It not only is more common than acute leukemia of either the myelogenous or the lymphocytic type but in a large series of collected cases has been found to be the most frequent of all the leukemias. It is about twice as common in males as in females.

Pathology

The pathologic features of chronic myelogenous leukemia are similar to those of the acute form. The enlargement of the spleen is greater, the consistency of the organ is more firm, and the capsule is thicker. The hematopoietic bone marrow is gelatinous in appearance and yellowish or pinkish



FIG. 57. Photograph of a section of normal bone marrow from the shaft of the femur. (Forkner, *Leukemia and Allied Disorders* The Macmillan Company)

gray in color. This cellular portion of the marrow is more extensive than normal and has encroached upon that portion which is ordinarily occupied by yellow or fatty marrow. The displacement of fatty marrow is more widespread in the chronic than in the acute form of the disease. The bone marrow on microscopic examination is found to be extremely cellular, and the

predominant cell is the myelocyte although the more immature myeloblasts as well as cells in all stages of development may be found (Figs 57 and 58). In certain instances erythropoietic hyperplasia is a striking feature. In others the megakaryocytes are particularly numerous. Eosinophilic myelocytes are

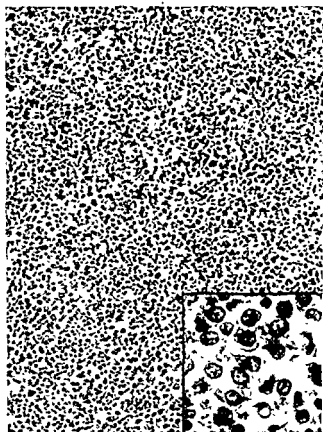


FIG. 58 Photograph of hyperplastic bone marrow from a case of myelogenous leukemia (Forkner, *Leukemia and Allied Disorders* The Macmillan Company)

sometimes very prominent in the histologic picture although the neutrophilic variety is usually the predominant type. Myeloid infiltration or metaplasia is extensive in the spleen and liver and occurs to a variable degree in other organs and tissues. The question as to whether the great numbers of myeloid cells in extramedullary locations occur as a result of infiltration of blood-borne cells or because of metaplasia and reversion of the reticulo-

endothelial tissues to their fetal function of cell production is debatable. It appears that the reticulo-endothelial cells retain the potential ability to form blood cells and revert to this function in myelogenous leukemia. Any organ or tissue may show these myeloid changes, but they are particularly striking in the organs which are rich in reticulo-endothelial tissue. However, the lymph nodes are not significantly involved until the late stages of the disease. The blood capillaries throughout the body are filled with myeloid cells, and there are linear or nodular collections outside the vessels.

An actual infiltration of the myocardium is occasionally encountered. An extensive infiltration of the lungs is sometimes present. The ovaries are not infrequently enlarged and show extensive cellular infiltration. The kidneys, adrenals, and other organs may be invaded. Hemorrhages into the various organs and under the serous surfaces are common in the late stages.

Clinical Features

The onset of chronic myelogenous leukemia is characteristically slow and insidious so that by the time a physician is consulted there may be a history of mild symptoms dating back a year or more. The most common mode of onset is a gradually progressive weakness, tiredness, and easy fatigability whereas other patients first notice a sense of weight and dragging in the left upper quadrant of the abdomen which results from the greatly enlarged spleen. A firm mass may be felt in this region by the patient before any symptoms are noticed.

During the course of the disease a wide variety of symptoms may appear, none of which are pathognomonic of myelogenous leukemia. Since the course is characterized by remissions and exacerbations, the intensity of the symptoms will vary from time to time. Weakness, loss of strength, fatigability, listlessness, and a gradual loss of weight occur in all cases, but their severity and the rapidity of their progression are variable. A progressive anemia is associated with the disease so that shortness of breath on exertion, pallor, tachycardia, and palpitation will be noted by many of the patients.

The gastrointestinal symptoms are likewise nonspecific in character. Many patients complain of a sense of fulness and distention, frequently of early satiety so that their hunger is appeased by small amounts of food, and they feel distended and unable to finish a meal. Nausea, vomiting, and gaseous eructations are occasionally noted although they are not apt to be troublesome until the late stages of the disease. The abdominal and gastrointestinal symptoms are surprisingly mild in many patients in whom the spleen is so

enormously enlarged that it would seem it should interfere more seriously with the functions of the other viscera.

Bleeding tendencies are prone to occur in the terminal stages of this disease. There may be severe hemorrhages from any mucous surface with epistaxis, melena, hematuria, or uterine hemorrhages. Bleeding from the gums as a result of slight trauma is very common even in the early stages, and many patients have noted that they have bruised easily for months or years. These slight bleeding tendencies are not necessarily associated with thrombopenia. Small hemorrhages are usually present in the ocular fundi, and occasionally extensive bleeding results in a loss of vision. Hemorrhage into the internal ear may cause deafness. Menorrhagia and other menstrual disturbances are not infrequent.

The enlarged spleen frequently causes a feeling of weight and fulness in the abdomen and may be the seat of severe, sharp pains which are intensified by respiration and are accompanied by a friction rub. These are due to infarctions of the spleen with a resultant perisplenitis. Although many patients do not experience this distress, it occurs repeatedly in others.

Pain and soreness of the bones is a not infrequent complaint in myelogenous leukemia. It is most commonly encountered over the lower sternum and ribs, but there may be transient and severe pains over any of the bones. Tenderness on deep pressure may or may not accompany the pain.

The basal metabolic rate is elevated, but the degree of elevation varies from time to time. A markedly raised metabolic rate is present during the acute exacerbations and is accompanied by profuse sweating, weakness, tachycardia, and intolerance to heat. A low grade fever may accompany the disease. During the acute phases the temperature may reach 103 or 104 F. Fever is a far less conspicuous feature in chronic than in acute myelogenous leukemia. Priapism is occasionally present. Shortness of breath and other symptoms referable to the circulatory system are common but occur more frequently as a result of the anemia than from actual cardiac involvement.

Physical Examination

The spleen becomes markedly enlarged, its size depends to some extent on the duration of the disease. It not infrequently extends below the brim of the pelvis and far to the right of the midline of the abdomen. It is very firm in consistency but maintains its normal configuration with a sharp medial border in which one or two notches may be felt. Occasionally it

causes a bulging of the abdominal wall below the costal margin. More frequently there is no visual evidence of the enlarged organ or only a slight fulness in this region. The size of the spleen may vary from time to time with the exacerbations and remissions of the disease or it may decrease during an intercurrent infection. The spleen may be tender, especially after in-



FIG. 59 Retina in a patient with acute myelogenous leukemia showing some edema of the nerve head, engorgement of the vessels, and extensive hemorrhage

fraction has occurred. There have been a few instances recorded in which there was no demonstrable splenomegaly.

The liver is enlarged in most patients, but this is less constant and less pronounced than the splenomegaly. The lymph nodes are not enlarged in the early stages of the disease, and most cases run their entire course without evident involvement. A few patients develop generalized lymphadenopathy, usually in the terminal stages. This signifies a generalized and extensive myeloid infiltration. In rare instances a marked lymphadenopathy has been encountered in the early stages.

Retinal hemorrhages are often encountered, usually small round or striate lesions which occasionally have a whitish center. Engorgement of the blood vessels is common, and edema of the nerve head occasionally occurs. Leukemic retinitis with leukocytic infiltration into the retina and along the course of the vessels may also be present (Figs. 59 and 60). Ménière's syndrome with deafness and vertigo may accompany hemorrhage into the labyrinth. Ascites is infrequent in myelogenous leukemia.

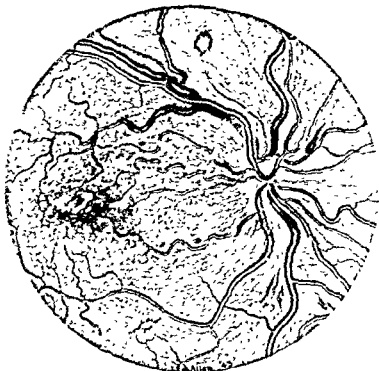


FIG. 60 Retina from a patient with myelogenous leukemia showing venous engorgement, edema of the nerve head, and small hemorrhages.

Purpura is of common occurrence, but leukemic infiltrations of the skin are far less common in myelogenous than in lymphocytic leukemia. When they occur they are usually small, flat, papular lesions with a dull reddish color (Fig. 61). They are of grave significance as they ordinarily appear only in the terminal stage. Lesions of the central nervous system are rare except for instances of cerebral hemorrhage.

Leukemic infiltration of the gums is frequent during acute exacerbations



FIG. 61. Leukemic skin infiltrations in a patient with chronic myelogenous leukemia.



FIG. 62. Osteoporosis, mottling, and perpendicular spicules of bone outside of the periosteum as found in a patient with aleukemic myelogenous leukemia (Baldrige and Fowler, *Arch Int. Med.*)

and in the terminal stages. The gums are spongy, bleed easily, and may be so swollen as to completely bury the teeth.

Although the urine shows albumin and casts, the renal function is not seriously impaired. Bence Jones protein is occasionally found in the urine. The excretion of uric acid is increased during the active phases of the disease, and the uric acid content of the blood may become elevated. Gout has been encountered as a complication of leukemia, and remissions of the leukemia have been observed to coincide with exacerbations of the gout. The blood cholesterol is frequently subnormal.



FIG 63. Roentgenogram of the hand of the same patient whose knee is shown in Figure 62. The perpendicular spicules on the metacarpal bones are very prominent.

Roentgenologic alterations in the skeletal system are encountered rather infrequently in chronic myelogenous leukemia. Some instances of localized cortical destruction, mottling of the bones, and generalized osteoporosis have been noted (Figs. 62 and 63). Wolf found 7 patients with osteosclerosis in which the blood picture was that of myelogenous leukemia.

Blood Picture

The total leukocyte count is extremely variable in chronic myelogenous leukemia. In the usual case it is several hundred thousand but not infrequently

it reaches 1,000,000 or more. Occasionally the leukocytes outnumber the erythrocytes. In an individual patient the count will fluctuate within wide limits in short periods of time and may drop to normal values spontaneously, as a result of an infection, or as a result of therapy. Spontaneous and persistent aleukemic phases are not infrequent so that the total leukocyte count is not of great significance in the prognosis except that a sudden increase or decrease is frequently a terminal event. An unexplained leukocytosis is the earliest manifestation of the disease in many, if not all, of the cases.

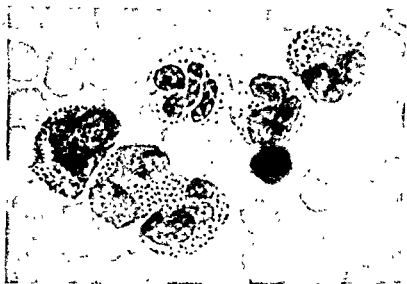


FIG. 64 Photomicrograph of blood smear from a case of myelogenous leukemia showing a group of myeloid cells ranging in maturity from a myelocyte to a segmented neutrophil. One nucleated erythrocyte is present.

In the differential count the predominant cell is of the myeloid or granulocytic series. All degrees of immaturity will be found (Fig. 64). The more acute and rapidly progressive the disease, the higher will be the percentage of immature cells. Myeloblasts are present during acute exacerbations but in slowly progressing and relatively quiescent stages they are infrequent. Myelocytes are the most characteristic cells. These may comprise from 10 to 90 per cent of the leukocytes, the higher values occurring in the more rapidly progressive cases. Metamyelocytes and nonsegmented polymorphonuclear neutrophils are also present in varying numbers. In very chronic cases the mature neutrophils and band neutrophils predominate on the smear, but as the disease increases in acuity these give way to more immature forms.

Neutrophilic granulocytes predominate in most cases, but eosinophils and

basophils may appear in large numbers either as mature or as immature forms. When high percentages of these cells appear, the disorder has been designated as eosinophilic or basophilic leukemia although it is merely a variant of ordinary myelogenous leukemia in which these cells rather than neutrophils predominate. Eosinophilic myelocytes are encountered in any case of myelogenous leukemia and are a valuable point in distinguishing myelogenous leukemia from a leukemoid reaction due to infection since eosinophils disappear from the blood stream in the presence of infection.

The percentage of lymphocytes is decreased, and but few monocytes are encountered. Care must be taken to distinguish lymphocytes from small myeloblasts (micromyeloblasts), and the monocytes must be distinguished from certain stages of the metamyelocyte. The blood smear of myelogenous leukemia presents an interesting and variable picture with cells in all stages of development appearing in the blood stream and all types of granules occurring in their cytoplasm. In the terminal stage or with the onset of an acute exacerbation a high percentage of immature cells appears, and the mature forms of the cells disappear from the blood stream.

Anemia is a constant feature of myelogenous leukemia, being mild at first but gradually progressing to a severe grade in the terminal stage. The color index is normal or slightly low, and only in the late stages of the disease do the erythrocytes appear unusually pale. Nucleated erythrocytes are commonly encountered on the smear, often appearing in showers.

The platelets are sometimes increased in number in the early stage but usually become markedly fewer as the disease progresses so that hemorrhagic features are common. Many large platelets are found in myelogenous leukemia. These may contain unusually large dark granulations.

Aleukemic myelosis or aleukemic myelogenous leukemia is a frequently encountered form of the disease in which the total leukocyte count is normal or low and immature cells are almost completely absent from the peripheral blood. This aleukemic phase may be temporary or permanent but is more common in the acute than in the chronic cases. The clinical features and the course are the same as in the leukemic type although roentgenologic changes in the bone and involvement of the skin appear to be more frequent.

The bone marrow obtained by sternal puncture reveals a hypercellularity as in the acute type but the cells are somewhat more mature. The bone marrow presents the same findings in both the leukemic and aleukemic forms.

Diagnosis

The diagnosis of chronic myelogenous leukemia is easily made in the typical case as the blood smear is characteristic. An elevated leukocyte count

with varying numbers of immature cells or an abnormal leukocytic reaction to an infection may precede the splenomegaly by many months. In such cases the disease must be differentiated from various types of leukemoid reactions.

The aleukemic form presents more difficulties in diagnosis, particularly in distinguishing it from aplastic anemia. The progressive anemia which does not respond to therapy, the splenomegaly, and an occasional immature myeloid cell in the peripheral blood are the principal features. Sternal puncture or biopsy may be necessary to establish the diagnosis.

Course and Prognosis

The rapidity of the course is extremely variable so that the disease may last for months or years. There are usually spontaneous remissions and relapses. The number of immature cells in the blood diminishes during a remission, and there is a remarkable subjective improvement which may last for months. Ultimately a relapse occurs and with this an accentuation of all the symptoms. As an acute episode develops, more immature cells appear in the blood stream. We have encountered many instances in which infection, trauma, or even the extraction of a tooth has been the factor initiating an acute exacerbation. Such features have also been found to precede the initial onset of the disease.

Pregnancy should be avoided by women with myelogenous leukemia since a severe relapse may be initiated by this condition. Most pregnancies terminate in premature labor, but in most instances no evidences of leukemia have been found in infants born to a leukemic mother.

The disease is invariably fatal, the average length of life is from three to four years. Rare instances in which the disease has been present for fifteen to twenty years have been recorded.

Treatment

Irradiation

The most widely used and most satisfactory method of treatment is irradiation. There is no uniform procedure for its use, and roentgenologists differ widely on the subject of proper voltage, amperage, and length of exposure. There is a diversity of opinion also as to the areas which should be irradiated. Some advise treatment only to the spleen. Others irradiate bones, spleen, and chest or use a form of spray therapy to the entire body. The spleen is ordinarily treated from the anterior, posterior, and lateral aspects (cross fire method) with adequate filters to protect the overlying skin

Irradiation of the bones must be used with caution since it may affect the erythropoietic tissues and so aggravate the anemia which is constantly associated with the disease. Irradiation of the entire body, with only the head, neck, and genital organs protected, has been used with gratifying results. This requires rather small but frequent exposures. The numerous treatments and the frequency of irradiation sickness are objections to the procedure. The results are only palliative and temporary regardless of the method of irradiation. It is doubtful if irradiation with extremely high voltages has any great advantage over that using lower voltages.

The response to irradiation varies from one person to another. The patient should be under close observation during the entire course of therapy and have daily leukocyte determinations. The leukocyte count can be reduced very rapidly by this form of therapy and continues to fall for several days after therapy has been stopped. Treatment should be discontinued before the count reaches normal as a severe leukopenia may otherwise develop. The most rapid reduction in the leukocyte count occurs in those patients with the highest initial count. The higher the percentage of immature cells, the more rapidly will it fall. A relatively low initial count or an aleukemic state is not a contraindication to therapy although a rapid decrease to a near normal level from a previously high level is an indication for cessation of treatment. A significant fall in the hemoglobin and erythrocyte levels during the course of therapy is also an indication to stop the irradiation as is a marked increase in the basal metabolic rate or the appearance of large numbers of immature cells.

The best response to irradiation is obtained during the first exposure, and progressively less effect is to be expected with subsequent therapy. Even the first course of treatment in some patients does not give satisfactory results. A favorable response produces a reduction of the leukocyte count to a point approaching the normal level and a reduction in the number of immature cells in the blood. The spleen recedes, in some instances a spleen which has extended to the brim of the pelvis may shrink beneath the costal margin. Coincident with these changes there is a marked subjective improvement and a return of strength, a lowering of the metabolic rate and a gain in weight. The duration of the remission varies with the acuity of the disease so that it may last for only a few weeks or may persist for years. Subsequent therapy should be given when the patient's symptoms return and should not be governed entirely by the leukocyte count. Increasing weakness, progressive enlargement of the spleen or liver, and increasing metabolic rate should be considered as indications for further therapy as well as a rising leukocyte

count. Roentgen ray therapy produces a beneficial result in a very high percentage of the patients. Comparisons between a series of treated and untreated patients show that this form of therapy prolongs their lives as well as producing a marked subjective improvement. Minot, Buckman, and Isaacs found that the duration of efficient life was 30 per cent longer in treated than in untreated patients.

Irradiation is not indicated in all patients with chronic myelogenous leukemia. In a few extremely chronic cases the symptoms are slight or negligible in spite of rather high leukocyte counts, and in such patients it is advisable to withhold therapy until the symptoms become more severe. Such cases are less common in chronic myelogenous than in chronic lymphocytic leukemia.

Irradiation sickness occurs in most instances in which intensive therapy is given over the spleen or liver. Irradiation of the bones is less apt to cause severe illness. Many measures and many drugs have been used to prevent or lessen this sickness, but none are successful.

When radium has been used in place of roentgen ray therapy, the results have been similar, but its use has been largely superseded by the roentgen ray. Radiophosphorus has been used effectively in the treatment of chronic myeloid leukemia and satisfactory remissions have been produced by this means although repeated transfusions may be necessary because the P^{32} delays the production of erythrocytes as well as leukocytes. Although the radiophosphorus may be given orally, it is usually given intravenously and repeated as frequently as necessary. It is given in the same manner as in polycythemia vera (see p 267). It does not induce irradiation sickness but the results do not appear to be any better than those obtained with roentgen irradiation.

Urethane

Experimental work has shown that ethyl phenylcarbamate and its derivatives exert a profound effect on the mitotic cycle of plant and animal cells. A suppressive action on the growth of certain bacteria, protozoa and plant and animal tissues has been noted and as a result the effects of these drugs have been studied in patients with certain forms of malignancies. They were found to produce a fall in the leukocyte count in such patients and were therefore used in the treatment of leukemia. The palliative effect has been very great in many cases although it is temporary and the patients have not been followed for a long enough period of time to evaluate the results satisfactorily.

ferential pattern approaches normal. The hemoglobin level tends to rise and

the spleen becomes smaller. There is a complete lack of response in some instances whereas in others a dramatic improvement results.

In lymphocytic leukemia the lymph nodes and spleen regress in size and the leukocyte count drops but the results are apparently less satisfactory than in myelogenous leukemia.

There is no indication that urethane is of permanent benefit in the leukemias nor has it been determined how long these remissions can be maintained. The use of this drug is in a purely experimental stage.

Other Therapeutic Measures

The nitrogen mustards have been used in myelogenous leukemia but the results are not as satisfactory as those obtained with roentgen irradiation.

Arsenic in the form of Fowler's solution has been successfully used both alone and in conjunction with irradiation therapy. It is best given continuously in small doses, beginning with a small amount, gradually increasing until evidences of toxicity appear, and then dropping to the small initial dose for continuous administration.

Benzol has been used but is dangerous, and the results are not as satisfactory as those obtained by irradiation or arsenic.

Thiouracil, which may produce agranulocytosis, is of no value in the treatment of leukemia even when given in very large amounts.

Transfusions of whole blood are effective in improving the patient's general condition and in relieving many of the subjective symptoms which accompany the anemia. The effect is transient, but they may prolong life in the terminal stage, hemorrhagic manifestations may be lessened, and the relief of the subjective manifestations may be striking. It is unwise to routinely transfuse all leukemic patients in the terminal stage of the disease, but the judicious use of transfusion in some cases is advisable.

The general measures for treatment include a high caloric and well balanced diet to maintain the patient's strength and nutritional state. The restriction of the patients' activities should be commensurate with their symptoms. They may be as active as their strength permits but should not tax their endurance beyond reason. There is no effective treatment for the accompanying anemia except the use of transfusions.

MONOCYTIC LEUKEMIA

There has been a great deal of confusion in the literature regarding monocytic leukemia. Much of it has arisen because of the differences of opinion which exist with regard to the origin of the monocyte. Recent studies sup-

port the idea that monocytes represent a separate series of cells and that monocytic leukemia is a clinical entity entirely separate from myelogenous and lymphocytic leukemia. If this view is correct, it is unnecessary to separate monocytic leukemia into the Naegeli and Schilling types, as is occasionally done, to indicate that the case in question is a modification of myelogenous leukemia (Naegeli type) or strictly monocytic in origin (Schilling type). The terms *reticulosis* and *reticulo-endotheliosis* have also been used synonymously with monocytic leukemia.

Monocytic leukemia is less frequent than either myelogenous or lymphocytic leukemia, representing only about 5 per cent of all cases of leukemia. It may occur at any age and has been reported in patients as young as 11 months and as old as 78 years. In our experience it has been more common in adults during the fifth and sixth decades of life and is more frequent in males than in females. It occurs in acute and chronic forms and may be leukemic or aleukemic in regard to the total leukocyte count. In this clinic the acute leukemic form has been by far the most common.

Pathology

Extensive leukemic infiltration of all organs, particularly of the spleen and lymph nodes, is found at autopsy. The predominant cell in the histologic picture is the monocyte. A widespread hyperplasia of monocytic cells is found in all connective tissue, and there is hyperplasia of the endothelium in the liver, spleen, lymph nodes, and bone marrow. Myeloid hyperplasia of the bone marrow is encountered in some cases.

Clinical Features

The clinical picture of monocytic leukemia is similar to that of other types of leukemia in most respects. There is a progressive anemia with the usual symptoms related thereto such as weakness, fatigability, shortness of breath, and palpitation. The metabolic rate is increased. Profuse perspiration, loss of weight, fever, and tachycardia are prominent symptoms. Hemorrhagic manifestations occur in most instances.

Swollen, spongy gums are encountered more frequently in monocytic than in other forms of leukemia. Another outstanding clinical feature is the necrotic ulceration which so frequently occurs in the buccal cavity and in the pharynx. The ulcers are sharply demarcated necrotic lesions which are surrounded by an extensive edematous infiltrated area with little or no purulent material. The swelling in these areas may be great enough to interfere with breathing or with swallowing. The regional lymph nodes may be extensively involved so that a large firm indurated mass will be apparent either within

the mouth and pharynx or presenting externally in the submaxillary region. Incision of the indurated area liberates only a small amount of serosanguineous material rather than pus. These lesions may be extremely painful.

Skin lesions also occur in a higher percentage of patients with monocytic leukemia than with the other types. There may be a diffuse exanthematous eruption with small macules and papules which become slate blue in color,



FIG. 65 Leukemic infiltrations of the skin in a patient with monocytic leukemia.

or the lesions may be large subcutaneous nodules which are firm, not tender, and of a dull reddish blue color (Fig 65). These may be so numerous as to be almost confluent and are especially common on the extremities. Small pustular-like lesions may also appear. They are usually transient, do not contain pus, and disappear without coming to a head. Microscopic examination of the lesions shows a leukemic infiltration in the deeper layers of the skin. When irradiation is applied there is a rapid disappearance of the lesions. Pain in the bones and joints has been noted, and transitory migrating pains of a "neuritic" type have been encountered in a few patients. No adequate explanation has been found for these.

The lymph nodes may or may not be enlarged. Palpable nodes were noted in only 77 per cent of the collected cases according to Osgood. The enlargement of the nodes, when present, is usually of moderate degree and not as extensive as that encountered in lymphocytic leukemia. The spleen is always found to be enlarged at autopsy, but this enlargement may not be sufficient to palpate during life or to appreciably increase the area of dulness. It seldom extends more than 2 or 3 cm. below the costal margin. The liver is usually enlarged but seldom excessively. A diffuse perivascular infiltration of the lungs may occasionally be detected on roentgenologic examination.

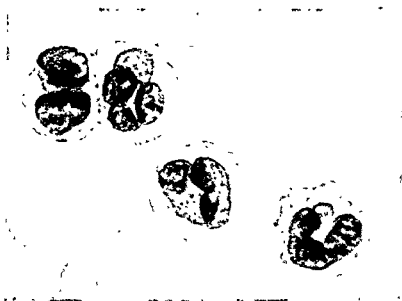


FIG. 66 Photomicrograph of a blood smear from a case of monocytic leukemia showing four immature monocytes with irregular folded nuclei

Blood Findings

A progressive anemia is encountered which becomes extremely severe in the terminal stage of the disease. The number of platelets is decreased in the late stages.

The total leukocyte count varies within wide limits. In 124 reported cases Osgood found that it ranged from 660 to 461,000 with an average of 99,600. There were 3 cases with persistent leukopenia and 6 which showed a leukopenia at some time during the course. On the smear the characteristic cell is the monocyte, which is found in varying stages of immaturity (Fig. 66). Monocytes comprise a large percentage of the leukocytes with the more

acute cases having a higher percentage of immature forms. Blast cells, indistinguishable from myeloblasts and lymphoblasts by their structural features, are present in small numbers, but in a majority of the cases the most numerous cells are those of the promonocyte stage of development. These are large cells with a basophilic cytoplasm containing many fine red dust-like granules. The nucleus is large and irregular in shape and appears to be lobulated. The chromatin network is so loose meshed that the nucleus is semitransparent and appears to be folded on itself since, because of the transparency, the outline of the underlying folds can be seen through the upper layer (Fig. 67). All shapes and configurations of the nucleus can be found, and nucleoli are frequent. Typical mature monocytes are likewise found in increased numbers. A few myelocytes are often encountered. As in the other leukemias the acute forms have a high percentage of immature cells in the blood stream and the chronic forms fewer immature but more mature monocytes. In the aleukemic phase immature cells are almost completely absent and the disease is extremely difficult to recognize.

The sternal marrow in monocytic leukemia shows a hypercellularity in which monocytes and promonocytes predominate. The morphology of the monoblast is so similar to that of the other types of blast cells that it cannot be distinguished with certainty and the recognition of the disease depends upon the presence of the more mature forms. These comprise a majority of the cells encountered. In no form of leukemia is the diagnosis so difficult as in aleukemic monocytic leukemia and sternal aspiration must be relied upon for a diagnosis in many instances. The abnormally large number of cells of the monocytic series, the presence of many cells containing mitotic figures, and a depression of the erythropoietic elements are the characteristic features.

Diagnosis

The diagnosis is based on the finding of immature monocytes in the blood stream and the presence of a progressive anemia. Monocytic leukemia can usually be differentiated from myelogenous and lymphocytic leukemia by examination of the blood smear, but sternal aspiration or biopsy of a lymph node may be necessary in some cases. An enormously enlarged spleen favors a diagnosis of myelogenous leukemia, and an extensive lymphadenopathy the lymphocytic type. Only a moderate or slight degree of splenomegaly and lymphadenopathy accompanies monocytic leukemia.

A leukemoid reaction of the monocytic type as a result of an infection is infrequent but may occur with infections of the gums, especially with

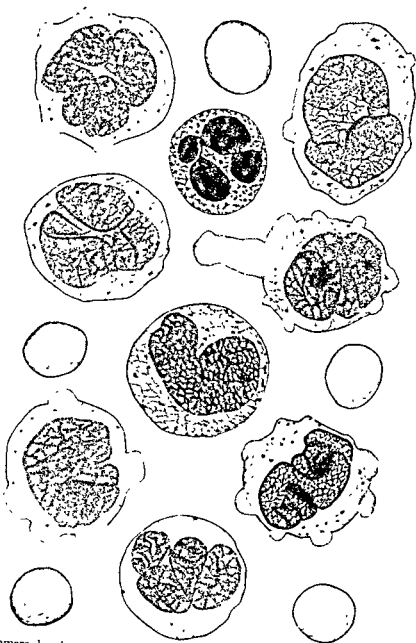


FIG. 67 Camera lucida drawings of monocytes from cases of monocytic leukemia. There are five erythrocytes for comparison as to size. In the upper center is a four-lobed neutrophil for comparison and below it a mature monocyte. The other seven cells are immature forms of the monocytic series showing the folded, irregular, and "transparent" nuclei. (Fowler, *J Lab & Clin Med*)

Vincent's angina We have encountered two cases, with an increased percentage of monocytes and few immature monocytes in the blood stream.

The course and prognosis of monocytic leukemia are similar to those of other leukemias. Most of the cases which we have encountered have been of the acute type, and Osgood found the average duration of life in 104 cases to be five and one-fourth months. The longest duration was four years. It appears that there is a greater tendency for this form of leukemia to be acute rather than chronic in its course. The disease is universally fatal.

Treatment

There is no satisfactory form of treatment. As in other acute leukemias irradiation is apt to do more harm than good although irradiation to the areas in the mouth which are the seat of necrotic ulceration and infiltration may relieve the swelling and pain and give considerable subjective relief. This does not influence the course of the disease. In the chronic forms irradiation by the spray technique should be used.

Transfusions may be used as a supportive measure but are of only temporary benefit. Other forms of symptomatic treatment should be used to make the patient as comfortable as possible.

CHLOROMA

Chloroma is a variant of acute myelogenous leukemia which is characterized not only by a leukemic blood picture but by the presence of invasive tumor-like growths of myeloid tissue. These growths usually arise from bones, most frequently from some part of the skull, but may occur in other organs and tissues. A greenish color is apparent on the cut surface of the fresh specimen, and it is from this color that the name "chloroma" is derived. The green color disappears upon exposure of the tissue to air although it may be restored by moistening the specimen with hydrogen peroxide. The disease is more common in males than in females and occurs predominantly in children and young adults.

The blood picture of chloroma is that of an acute myelogenous leukemia. Only occasionally does it suggest the chronic form of the disease. A majority of the cells encountered on the smear are myeloblasts and myelocytes, and only a few are mature cells. We have observed one patient in whom Auer bodies (small red-staining rods) were present in 50 per cent of the cells, a majority in the promyelocytic stage without specific granulations.

The general symptoms are those of any acute form of leukemia with weakness, fever, palpitation, elevated basal metabolic rate, loss of weight, and a progressively downward course.

The tumor-like masses of myeloid cells have a special predilection for arising from the bones of the skull, the sternum, ribs, and vertebrae and in these locations produce symptoms by their pressure on various structures. Exophthalmus is a common feature of the disease when the bones about the orbit are involved. The inner ear or the eighth nerve may be affected when the temporal bones are involved; deafness may result. The tumors are usually multiple and have a tendency to invade the periosteum and dura so that many neurologic complications may result from cranial or vertebral lesions. There may be pain, paralysis, or weakness of the extremities, incontinence, and evidences of myelitis or of involvement of the cranial nerves. Periosteal swelling may occur in any bone. Osteoporosis is a common feature, and pathologic fractures may occur. Doek and Warthin collected 22 cases and found the following incidence of bone involvement: orbits 12, sinuses 9, temporal 7, temporal fossa 8, sphenoid 3, ethmoid 2, vertebra 9, sternum 7, ribs 8, as well as the sacrum, coccyx, iliac bones, extremities, and other short bones.

In addition to the involvement of periosteum and bone these greenish tumors have been found in the kidneys, liver, spleen, lymph nodes, ovaries, and other organs

The histologic picture reveals an invasive tumor mass composed of immature myeloid cells which destroy the substance and structure of the bone. Although it affects predominantly bone and periosteum, other organs may be studded with small greenish colored tumors

The course is that of acute leukemia. Duration of life is usually a matter of a few weeks or months

Irradiation therapy may not influence the ultimate outcome of the disease but it does decrease the size of the tumor masses and relieves the pain and other pressure symptoms. There have been two cases of apparent cure following irradiation therapy so that intensive and prolonged therapy is advisable even though most cases do not show such a favorable response

PLASMA CELL LEUKEMIA

There has been some question as to the existence of plasma cell leukemia as a distinct clinical entity since the origin of this particular cell is not well established. The general opinion has been that the plasma cell is a member

of the lymphocytic series and that plasma cell leukemia occurs either as a variant of lymphocytic leukemia or as an unusual blood reaction in cases of multiple myeloma.

A majority of the cases of multiple myeloma are of the plasma cell type (plasmacytoma). In most instances there are no significant changes in the leukocytes of the peripheral blood. Occasionally in this disease plasma cells are found in the peripheral blood in sufficient numbers to suggest leukemia, and this may be accompanied by an infiltration of the various organs.

Osgood and Hunter record a case in which the leukocyte count was 24,200 with 54 per cent plasma cells. No lesions of the bone were found. There was enlargement of the spleen and lymph nodes, and the clinical course was more suggestive of leukemia than of multiple myeloma. The predominating leukocyte in the blood stream was round or oval in shape with an eccentric nucleus which had a coarse chromatin structure and dense masses of chromatin at the periphery. In their opinion the origin of the plasma cell is entirely separate from the lymphocyte. They feel that plasma cell leukemia should be considered as a distinct entity. Similar cases have been described, but a high plasma cell count in the peripheral blood is more frequently associated with multiple myeloma. Occasionally the hematologic features suggested plasma cell leukemia when first observed but ultimately developed the typical features of lymphocytic leukemia.

Whether or not plasma cell leukemia constitutes a clinical entity, a generalized or leukemic phase of plasmacytoma, or a form of lymphocytic leukemia cannot be definitely settled at the present time but there is increasing evidence that plasma cell leukemia constitutes a clinical entity separate and distinct from multiple myeloma.

EOSINOPHILIC LEUKEMIA

Eosinophilic leukemia is a variety of myelogenous leukemia in which eosinophils and eosinophilic myelocytes are unusually prominent in the blood stream. This condition may occur in either the acute or the chronic form of the disease, and the symptoms, findings, and course differ in no way from those of ordinary myelogenous leukemia, in which the neutrophilic strain of cells predominates. In most cases of myelogenous leukemia there is both an actual and a relative increase in the number of eosinophils in the blood stream. Not only are eosinophilic myelocytes encountered in the peripheral blood but their number is markedly increased in the bone marrow. Eosino-

phils in various stages of growth have been found to account for 50 to 80 per cent of the nucleated cells of the blood stream in some cases of myelogenous leukemia. It is this type of case that has been termed eosinophilic leukemia. Since this is merely a variant of myelogenous leukemia, it would seem best to avoid confusion and drop the term eosinophilic leukemia.

The presence of eosinophilic myelocytes in the blood stream is often of definite value in arriving at a diagnosis of myelogenous leukemia. Eosinophils characteristically disappear from the blood stream in the presence of an infection so that the appearance of eosinophilic myelocytes aids in excluding a leukemoid reaction due to infection.

BASOPHILIC (MAST CELL) LEUKEMIA

Certain cases of myelogenous leukemia show a great increase in the percentage of basophils in the peripheral blood. Examination of the bone marrow reveals excessive proliferation of basophilic myelocytes. These have been reported as examples of basophilic or mast cell leukemia although they are merely variants of myelogenous leukemia, the only condition with an increased number of basophils and basophilic myelocytes in the peripheral blood stream. The presence of these cells is an aid in distinguishing myelogenous leukemia from leukemoid reactions due to infection.

MEGAKARYOCYTIC LEUKEMIA

Megakaryocytes are occasionally found in the blood stream in patients with Hodgkin's disease, polycythemia vera, and myelogenous leukemia. In the latter condition they occasionally appear in fairly large numbers and have comprised as high as 18 per cent of the nucleated cells. Such cases have been reported as megakaryocytic leukemia but it is probable that this is only a variant of myelogenous leukemia.

AGNOGENIC MYELOID METAPLASIA OF THE SPLEEN

Agnogenic myeloid metaplasia of the spleen is characterized by a progressive enlargement of the spleen and by the presence of immature erythrocytes and leukocytes in the blood stream. The principal symptoms are weakness, abdominal distress, and hemorrhagic tendencies. The leukocyte count is variable and may show either a moderate elevation or a slight leukopenia.

The percentage of band neutrophils is increased, and a few myelocytes and metamyelocytes are found, with varying numbers of nucleated erythrocytes. A moderate or severe anemia is a constant finding. Icterus may be present, but the fragility of the erythrocytes is normal, and there is no spherocytosis. Examination of the sternal marrow does not show a leukemia infiltration.

Histologic examination of the spleen shows scattered foci of hematopoiesis with immature erythrocytes, leukocytes, and megakaryocytes. Similar foci are encountered in the liver and lymph nodes, but there is not the uniform distribution and infiltration of immature cells that is found in leukemia. Although the bone marrow may show either fibrosis, hyperplasia, or aplasia, its condition is not suggestive of leukemia.

The course of the disease is chronic. The average duration of life in the reported cases was ten years after the appearance of symptoms with 4 of the patients having symptoms for fifteen years. It must be distinguished from hemolytic icterus, Banti's syndrome, and myelogenous leukemia.

Both irradiation of the spleen and splenectomy are contraindicated as the condition becomes worse after these procedures. There is no treatment known to be of benefit except supportive measures.

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Chapter XVIII

LYMPHOMAS

THE LYMPHOMAS COMPRISE A GROUP OF PATHOLOGIC AND CLINICAL ENTITIES which are characterized by progressive, painless enlargement of the fixed lymphatic tissues and fatal termination. They are the subject of much dispute among both pathologists and clinicians as to their etiology and classification. Any classification which is adopted at the present time or any terminology which is used will be subject to criticism. The clinical manifestations of the various conditions included under the term *lymphoma* are so similar that, in the absence of specific changes in the circulating blood, their differentiation is frequently impossible except by microscopic examination of a lymph node. The histologic picture is usually sufficiently distinctive to make the various types of the disease recognizable, but occasionally pathologists differ in their interpretation. Although we prefer to think of these diseases as being neoplastic in origin and use the term *malignant lymphoma* in referring to them, the possibility of an infectious origin cannot be denied. The lymphomas are separated into Hodgkin's disease or the sclerosing type of lymphoma, the lymphocytic type, the lymphoblastic type or lymphosarcoma, and the follicular type.

HODGKIN'S DISEASE

Hodgkin's disease is a condition characterized by progressive enlargement of the fixed lymphatic tissues which causes systemic manifestations as well as pressure symptoms from the enlarged lymph nodes. The course is progressively downward with ultimate cachexia and a fatal termination. This disease is also known as lymphogranuloma, lymphogranulomatosis, and as the sclerosing type of lymphoma.

Hodgkin's disease is widespread throughout the world and does not appear to be unduly prevalent in any one locality or country or among any one particular race of people. There is no hereditary or familial predisposi-

tion, and occupation plays no part in its incidence. It is primarily a disease of early adult life, being most common in the third and fourth decades. No age is exempt, however, as it has been encountered in children as well as in adults over 80 years of age. It is two or three times as common in males as in females.

The cause of Hodgkin's disease has not been ascertained. The histologic appearance of the involved lymph nodes resembles an infectious granuloma in many respects, but the course of the disease is more suggestive of a neoplastic lesion. The numerous attempts to reproduce and to transmit the disease have all failed as have attempts to demonstrate a causative organism. Although the disease has been thought to be an atypical form of tuberculosis, or due to avian tuberculosis, there has been no convincing proof of these contentions. It has also been blamed on a diphtheroid bacillus, a *Brucella* infection, and a virus infection, but adequate proof has not been forthcoming to definitely incriminate any of these organisms. Although a substance obtained from a node involved by Hodgkin's disease has been found to produce encephalitis in experimental animals, subsequent investigations have shown that the same response can be obtained with extracts from any tissue containing eosinophils.

Many pathologists believe that the histologic picture is more in keeping with a neoplastic disease than an infectious process. The clinical course with its invariably fatal outcome tends to substantiate this belief. The present consensus in this country favors the neoplastic theory of origin, but this must be left an open question at present.

Pathology

The characteristics of the involved lymph nodes vary a great deal with the stage of the disease at which the examination is made. Each node is round or oval in the early stages, the surface is smooth, there is no invasion of the capsule and no periadenitis. The consistency of the node is elastic and rather soft whereas in the later stages it becomes firm and hard. The color varies from grayish white to pink. The early histologic changes consist of hyperplasia of the lymphoid elements, proliferation of the germinal centers, and dilatation of the lymph sinuses. There is also proliferation of the supporting reticulum, and with this expansive growth the normal architecture of the node is displaced, the hilus pressed out, and the capsule stretched. In later stages the node becomes sclerotic with the normal structure entirely replaced by connective tissue proliferation. The reticular network becomes more prominent, coarse strands of fibrous tissue appear, and within the meshes

of this network are lymphocytes, epithelial cells, and plasma cells together with many eosinophils and giant cells. The polynucleated giant cells—Dorothy Reed giant cells—form a conspicuous feature in the histologic pic-



FIG. 68 Hodgkin's disease. A large mass of cervical nodes is present on the right, and pressure on the sympathetic nerves has produced Horner's syndrome. The ptosis of the right lid with a narrowing of the palpebral fissure is evident in the photograph.

ture. Lymphoid tissue, wherever it is found in the body, may be the seat of these pathologic changes, and nodules have been found in almost every tissue and structure of the body. The spleen is usually enlarged; on cut section the pulp is red with many small irregular white or yellowish masses. Infarctions of this organ are frequently encountered.

Clinical Features

The manifestations of Hodgkin's disease are so variable that it is impossible to describe anything which might be called a typical course. The systemic manifestations, the pressure symptoms, and the symptoms arising from invasion of various tissues tend to produce a syndrome which may simulate almost any disease and be referred to any part of the body.

The most conspicuous feature, but by no means an invariable manifesta-

tion, is lymphadenopathy. This is usually the patient's presenting complaint and commonly makes its first appearance in the cervical region. The nodes are small at first but gradually increase in size and number. They are usually not tender or painful so that they occasionally become quite large before they are noticed by the patient. In many instances there are periods of spontaneous regression during which previously enlarged nodes become smaller or shrink to their normal size only to enlarge again after a variable interval. They may appear first on one side of the neck and later spread to the opposite side, or there may be a generalized involvement by the time they are first detected. The nodes are smooth, discrete, not tender, and freely movable, and the overlying skin is uninvolved. In the early stage they are firm but rather elastic in consistency. As the disease progresses the adenopathy extends along the lymphatic chains and the nodes become more numerous and

larger (Fig. 68). A large pyramid-shaped mass of nodes frequently develops in the cervical region with its base at the clavicle and its apex directed upward.

The axillary, inguinal, and femoral nodes become enlarged. Their involvement may precede or follow the appearance of cervical adenopathy. These nodes have the same characteristics as those in the cervical region but usually do not become as large. Epitrochlear nodes, subclavicular nodes, as well as nodes in the most unusual locations may ultimately become palpable. The typically discrete and movable nodes of Hodgkin's disease become matted

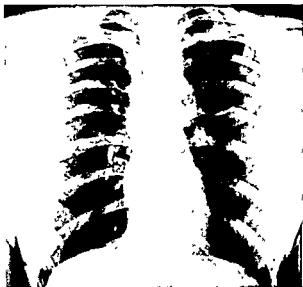


FIG. 69 Hodgkin's disease involving the mediastinal nodes, showing a single node of medium size.

together and anchored only in the late stage of the illness. Although they are rather soft in the early phase of the disease, they later become extremely hard in consistency. Suppuration of the glands has been observed but rarely. Involvement of the superficial nodes may be slight or absent with the greatest enlargement being found elsewhere. The mediastinal nodes may be enlarged to varying degrees either alone or in conjunction with involvement of nodes in other locations (Figs 69 and 70). The abdominal nodes, either mesenteric or retroperitoneal, may become enlarged and form irregular tumor masses. The detection of enlarged abdominal nodes is more difficult than of nodes in other parts of the body so that their involvement is recognized less fre-

quently. In certain instances, however, the abdominal lymphadenopathy is the most outstanding feature.

The lymphadenopathy in Hodgkin's disease is not uniform. Extremely large nodes may be present in one location while only small ones will be found elsewhere. The tonsils are infrequently involved although involvement of extranodal lymphoid tissue elsewhere in the body is not uncommon.

The spleen is enlarged in practically all cases which come to necropsy, but the splenomegaly may be so slight as to be undetected during life. The



FIG 70. Hodgkin's disease, showing a massive involvement of the mediastinal nodes

organ is palpable in about 75 per cent of the cases, but the enlargement is usually moderate in degree. Extreme grades of splenomegaly are not common. In some instances splenomegaly precedes the lymphadenopathy or it may be present with only slight enlargement of other nodes. Although such cases have been termed the "splenic type" of Hodgkin's disease, it is doubtful if the spleen is ever the only site of involvement. In the "abdominal type" there is splenomegaly and involvement of the abdominal nodes with absent or minimal involvement in other regions.

Pressure Symptoms

Pressure from the enlarged nodes may be exerted on the neighboring structures with production of a wide variety of symptoms. Enlarged cervical nodes may press upon the cervical sympathetics to produce Horner's syn-

drome, unilateral ptosis of the upper lid, enophthalmus, myosis, and anhidrosis on the affected side. In other instances pressure of the enlarged cervical nodes causes severe pain

Extensive involvement of the mediastinal nodes is prone to produce alarming and troublesome symptoms due to pressure on the trachea and esophagus



FIG. 71 Engorgement of the veins in the neck and chest as a result of the pressure of enlarged mediastinal nodes in Hodgkin's disease—

as well as on vessels and nerves. Dyspnea, stridor, and a distressing cough may be caused by tracheal obstruction, and dysphagia results from pressure on the esophagus. Cyanosis and engorgement of the veins of the neck and upper chest wall result from obstruction of the superior vena cava (Fig. 71). Pressure on the recurrent laryngeal nerve may cause paralysis of the vocal cords, and pressure on the vagus may produce tachycardia. Atelectasis of a portion of the lung with secondary infection and cavity formation may fol-

low occlusion of a bronchus. Pleural effusion may result from venous obstruction and in some instances may be accompanied by ascites and edema of the lower extremities. A chylous effusion may result from pressure on the thoracic duct.

Pressure of nodes within the abdominal cavity may lead to various gastrointestinal symptoms such as dyspepsia, constipation, and early satiety. Jaundice may result from pressure on the common bile duct. Pressure on the kidney and urinary tract has not been common in our experience although pain, urinary retention, hematuria, and pyuria have been reported.

Enlargement of the inguinal and femoral nodes may interfere with the venous return from the lower extremities and so lead to edema.

Infiltrative Lesions

Infiltration of any organ containing lymphoid tissue may occur in Hodgkin's disease. The gastrointestinal tract is not infrequently involved with thickening, induration, and infiltration of the walls. Such involvement seldom occurs in the lower bowel, being more frequent in the stomach and upper part of the tract. This infiltration may cause a filling defect and a thickening of the wall of the stomach which suggests gastric carcinoma on roentgenologic examination. We have observed one case in which roentgen ray studies failed to reveal an infiltrative lesion which could be seen through the gastroscope. Gastrointestinal symptoms are common as a part of the systemic reaction to Hodgkin's disease whereas actual infiltration and pressure symptoms are relatively infrequent. It cannot be assumed that all patients with dyspeptic and digestive symptoms have actual lesions in the intestinal tract.

Infiltration of the lung has been encountered in as high as 50 per cent of autopsied cases. This is more frequent in patients in whom the mediastinal nodes are involved but is not a simple compression from this enlargement. The infiltration may be slight and nodular in type, or there may be an extensive lobular involvement with symptoms and findings suggestive of tuberculous pneumonia. Pleural effusion may be due to venous or lymphatic obstruction from enlarged mediastinal nodes, but actual invasion of the pleura by lymphomatous tissue may also cause it.

Bone is frequently invaded—in 24 per cent of one series of cases of Hodgkin's disease. The type of lesion which results is variable. It may be either destructive or proliferative, both types having been observed in some patients. Almost any bone may be involved although the flat, hematopoietic bones are invaded most frequently. Vertebral involvement may cause collapse of the vertebra with pressure on spinal cord or nerve roots. Severe pain

over the involved area is suggestive of osteomyelitis in some instances, in other cases the invasion of bone is symptomless. A particularly severe grade of myelophthisic anemia develops when the involvement is extensive in the skull, pelvis, or other hematopoietic bones.

Hodgkin's disease frequently involves the skin in many different ways. Intense itching is a very common complaint. It is frequently generalized and unassociated with any obvious histologic alteration in the skin. This may be the initial complaint, preceding other evidences of the disease by a considerable period of time. The skin is usually dry and rough with inflammatory thickening from the continued scratching. In a few instances there is a diffuse infiltration of the skin, or there may be a nodular type of involvement with small or large tumor masses. Herpes zoster is a frequent complication.

Invasion and destruction of the adrenal glands with the development of a typical clinical picture of Addison's disease have been noted.

Systemic Manifestations

The systemic manifestations of Hodgkin's disease are variable and may appear either before or after the lymph nodes become enlarged. In some instances an unexplained fever with malaise, profuse perspiration, and loss of weight appears before the lymphadenopathy. In even more infrequent instances, these are the only obvious manifestations of the disease. Fever and prostration are apt to be more severe in those patients in whom the spleen and abdominal nodes are the primary site of the disease. The lymphadenopathy is usually discovered before systemic manifestations make their appearance. Ordinarily it is only after lymphadenopathy has been present for several weeks that the febrile reaction occurs. Fever is probably present in all patients at some time in the course of the disease. It may be continuous or intermittent. The most characteristic febrile course is the Pel-Ebstein type, in which there are alternating febrile and afebrile periods. The temperature gradually increases, frequently to 103 or 104 F., and persists at this level for one to three weeks, then subsides to normal for a variable period only to rise again and repeat the cycle. Chills, lassitude, and profuse perspiration are noted during the febrile periods.

The constitutional symptoms are more severe in the acute, rapidly progressive cases and in the terminal stage of the chronic forms. The patient may go for a long period of time without significant weight loss or there may be a progressive loss beginning soon after the onset of the disease. The basal metabolic rate is usually increased.

Blood Findings

A moderate degree of anemia appears relatively early in the course of most cases of Hodgkin's disease. In the acute form of the disease the anemia is progressive and parallels the course whereas in the chronic form it remains stationary or is very slowly progressive until the terminal stage when it becomes severe. A particularly severe grade of anemia of the myelophthisic type develops when there is extensive involvement of the bones. There are no significant changes in the erythrocytes except for slight hypochromia although occasional nucleated erythrocytes may be found on the smear when the bone marrow is invaded.

The leukocyte count may be low, normal, or elevated, leukocytosis being common and leukopenia rare. Leukocyte counts of 20,000 to 25,000 are not uncommon although 10,000 to 15,000 is the usual range. The count may vary greatly from day to day with no apparent reason. There is no correlation between the stage of the disease and the total leukocyte count.

The differential count is also variable. The most common finding in the late stage of the disease is an increase in the number of neutrophils. These frequently comprise from 85 to 90 per cent of the white blood cells. An eosinophilia is infrequent but may occur in the late stage and occasionally is a striking feature. We have encountered one case in which 85 per cent of the leukocytes were eosinophils. The number of monocytes is occasionally increased to a moderate degree in the earlier stages of the disease, and a slight lymphocytosis with some abnormal forms has also been encountered during the period when the lymph nodes were rapidly enlarging.

Course

The course of the disease may be acute and rapidly progressive or very chronic. In a few cases the disease begins with fever and malaise associated with moderate lymphadenopathy and pursues a very rapid course with progressive anemia, weakness, and cachexia. The entire course may last but a few months. In other instances the lymphadenopathy appears before the systemic manifestations, the size of the nodes increases slowly, and the course is very chronic so that the disease has been known to go on for as long as fifteen years or more after the onset of symptoms. The late stages are characterized by weakness, loss of weight, and cachexia, and the picture is like that of a malignant growth. The course may be complicated at any time by pressure on various structures or organs. Death may be due directly to this. The average duration of life after the onset of symptoms is from three to four years.

Diagnosis

The diagnosis of Hodgkin's disease is difficult or impossible without histologic examination of a lymph node. A presumptive diagnosis can be made in the presence of a generalized lymphadenopathy with the characteristic discrete smooth nodes, splenomegaly, and Pel-Ebstein fever, but a similar clinical picture may be produced by other diseases. Final proof of the diagnosis lies in biopsy of a node.

Tuberculous lymphadenopathy has frequently been confused with Hodgkin's disease although the glands have a greater tendency to become fused together, to involve the overlying skin, to suppurate, and to remain more localized. In the absence of these distinguishing features a biopsy may be necessary for differentiation.

Lymphocytic leukemia, the lymphocytic type of lymphoma, will produce a generalized lymphadenopathy but can be recognized by the blood findings. Aleukemic lymphocytic leukemia causes a generalized lymphadenopathy in which the nodes are quite uniform in size. There are no distinctive clinical features, and the final interpretation will depend on biopsy.

Other types of lymphoma, such as the lymphoblastic type and the follicular type, are so similar to Hodgkin's disease that an absolute differentiation is impossible without histologic examination of a lymph node.

Carcinoma of the nasopharynx may give rise to cervical lymphadenopathy that simulates Hodgkin's disease although the nodes are very hard even when they first make their appearance.

Boeck's sarcoid may involve the skin, mediastinal nodes, lungs, and bones and presents a picture resembling Hodgkin's disease so that the differentiation by clinical means is difficult.

Lupus erythematosus disseminatus may occasionally present a picture resembling Hodgkin's disease, but lymphadenopathy is not a prominent feature.

Infectious mononucleosis or glandular fever may cause generalized lymphadenopathy. It is accompanied by fever, sore throat, and usually splenomegaly, but the other symptoms are extremely variable. The blood smear is usually diagnostic with many abnormal and degenerated lymphocytes. If this feature is indeterminate, the heterophile antibody test may establish the diagnosis.

Infections of various types may cause generalized lymphadenopathy, among the most common being secondary syphilis, German measles, plague, tuberculosis, infectious arthritis in children (Still's disease), or a generalized dermatitis.

Localized lymphadenopathy is usually due to an infectious process or a metastatic malignant tumor in the area drained by the involved nodes.

Treatment

The best therapeutic results are obtained by irradiation therapy. This may be given only to the obviously involved lymph nodes or to other areas of lymphoid tissue which are suspected of being involved. The total amount of irradiation as well as the amount which is given with each treatment must be individualized so that an effective dosage will be administered but in small enough doses to be well tolerated. The erythrocyte and leukocyte counts should be followed closely during the course of treatment so as to prevent a severe anemia or leukopenia from developing. A dose of 600 r is commonly applied to superficial lesions whereas 1200 to 1500 r may be given to deep-seated lesions.

The response to irradiation therapy is frequently dramatic. Large masses of lymph nodes may rapidly subside to normal or near normal size, and large mediastinal masses may shrink to the point that no evidence of enlargement remains on roentgenologic examination (Fig. 72). Pressure symptoms rapidly subside as the size of the glands diminishes, but it must be borne in mind that immediately after therapy there may be some increase in the size of the nodes with a temporary accentuation of symptoms. This is particularly important when there is partial tracheal occlusion as in this location the additional swelling may cause alarming symptoms or even asphyxia. The most rapid response to therapy is obtained in the early stage of the disease when the glands are soft in consistency and contain many lymphocytes which are sensitive to irradiation. The response is less striking when sclerosis and scarring are present in the glands. Progressively less effect is obtained with repeated courses of therapy.

The duration of the beneficial effect is variable. Months or years may elapse before further treatment is necessary in some cases; in others it is only a matter of a few weeks. Treatment should be repeated when there is evidence of recurrence. Irradiation will frequently control the pain associated with lesions in the bones and may cause a marked regression of the infiltrative lesions in the lungs. In the later stages of the disease the intervals between courses of irradiation therapy will become shorter.

The effect of irradiation on very early localized lymphadenopathy without pressure symptoms or constitutional manifestations has not been adequately studied. There are some who believe that the ultimate course of the disease is unchanged by irradiation and that therapy should be reserved until pres-

sure symptoms or markedly enlarged glands are present. Others feel that irradiation of nodes before the involvement becomes generalized may stop or markedly retard the course of the disease even though an actual cure may not be obtained. The latter view seems the most logical one to accept at present. The survival period is apparently longer in treated than in untreated cases even though a complete cure is not obtained.

Radium may be used in place of roentgen irradiation, particularly for

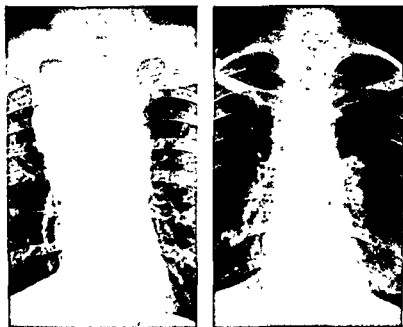


FIG 72 Hodgkin's disease of the mediastinum. The figure on the left shows the size of the mediastinal shadow before roentgen ray therapy. Right, the mediastinum of the same patient after therapy.

localized and superficial lesions, but its use has been largely replaced by roentgen ray therapy.

Surgical removal of the affected nodes has been attempted when the disease is in an early and localized stage. It is doubtful, however, if all the affected nodes can be removed so that, rather than being cured, the disease is merely retarded to some extent. Although apparent cures by surgical treatment have been reported, the diagnosis in such cases is doubtful.

Fowler's solution, which has been tried in Hodgkin's disease, has been largely replaced by irradiation therapy. It is still used to some extent in conjunction with irradiation although its value is questionable.

General symptomatic treatment should include a high caloric and high

vitamin diet. Transfusions may be used to counteract the anemia as this does not respond to other forms of therapy.

Nitrogen Mustard Therapy

The nitrogen mustards, tris (β -chloroethyl) amine hydrochloride and methyl-bis (β -chloroethyl) amine hydrochloride developed as contact vesicants during World War II were found to have a toxic action on cells which is very similar to that of roentgen rays. The effect of these drugs are most pronounced on the hematopoietic tissues, bone marrow and lymphoid tissue, but also are active against any rapidly growing and proliferating tissue, causing fragmentation, degeneration, loss of mitotic activity, and ultimately atrophy of the tissue. These drugs are still in an experimental stage and it is to be hoped that more effective and less toxic compounds of this type may be developed. The compounds are administered intravenously in a dosage of 0.1 mg. per Kg. of body weight with a series of four to six such injections being given at twelve to twenty-four hour intervals. Such courses may be repeated as often and for as long a period of time as the hematologic status of the patient permits, usually allowing a four-week interval between courses. The material is obtained in dry form in sterile tubes and is dissolved by injecting 10 cc. of sterile saline into the original container and withdrawing the amount of solution required. It must be administered within five minutes after its preparation. The best method of administration is by injection of the material into the rubber tubing of an intravenous medication set through which isotonic saline is already running. The drug must be given with extreme caution since it is extremely irritating to the skin and tissues and is also liable to cause thrombosis within the vein.

The toxic effects of methyl-bis (β -chloroethyl) amine hydrochloride, according to Rhoads, consist of (1) a severe local inflammatory reaction if the material escapes into the tissues at the site of injection, (2) varying degrees of nausea and vomiting occurring one to eight hours after the injection and lasting three to twenty-four hours, associated with anorexia, weight loss, weakness, and headaches, and (3) damage to the blood-forming organs. The alterations in the peripheral blood which may result are. (1) Leukopenia which may be moderate to severe and may persist for a month or more. This is first a lymphopenia but is followed by a neutropenia. (2) Normocytic anemia is not infrequent. (3) Bleeding tendencies are rare.

The therapeutic value of nitrogen mustards, according to the official statement, is: (1) They are not a cure for such neoplastic diseases as have been studied. (2) The nitrogen mustards in large enough doses are injurious to

many types of tissue, they appear to exert their greatest effects on rapidly growing tissue, presumably either normal or neoplastic. (3) Their predominant toxicologic effect is damage to normal hemopoietic function. The extent of this injury is the limiting factor in determining the amount that can be given to an individual. In some cases the hemopoietic injury exceeds the effect on the tumor. (4) The tumor regressions induced by these compounds (even with maximum dosages) are temporary, with maximal persistence rarely extending beyond several months. (5) The effects of the nitrogen mustards are in many respects similar to those of roentgen rays. It should be noted, however, that the great advantage of radiation therapy is that it can be given locally.

Methyl-bis (β -chloroethyl) amine hydrochloride has proven to be more effective in the treatment of Hodgkin's disease than in any other type of lymphoma or neoplasm. In many instances a marked regression in the size of the nodes has been observed together with a disappearance of the constitutional and subjective manifestations. Such remissions are temporary, however, lasting for a few weeks or months at the most. It has frequently been noted that patients who have become resistant to roentgen-ray therapy may again respond to irradiation after one or two courses of nitrogen mustard. We have found it most useful in patients in whom the lymphadenopathy is well controlled by irradiation therapy but who continue to complain of weakness, fever, and night sweats.

Because of the effects on the erythropoietic elements an anemia is prone to develop three to four weeks after a course of treatment and transfusions may be necessary at this time. A subsequent spontaneous improvement in the erythrocyte count may occur. A leukopenia develops as a result of the nitrogen mustard therapy, but even though the leukocyte count drops to a low level infections seldom develop and a spontaneous increase in the leukocyte count occurs. A thrombocytopenia also occurs and hemorrhagic tendencies may develop. The height of the anemia, leukopenia and thrombocytopenia usually occur about three to four weeks after treatment.

The remissions produced by nitrogen mustard tend, as with roentgen-ray therapy, to become shorter and shorter with subsequent courses. In some instances the best results are obtained with a combination of, or alternating courses of, nitrogen mustard and irradiation. The drugs at the best are palliative, not curative, but do provide a means of temporarily arresting the progress of the disease in some cases and have produced gratifying amelioration of symptoms at times.

Equally good results, but of shorter duration, have been obtained in some

patients with lymphosarcoma. In this disease, as well as in Hodgkin's disease, the best results are obtained in the more chronic and slowly progressing forms while in those with a more rapid course the results are less gratifying.

The results obtained with the nitrogen mustards in chronic lymphatic leukemia are similar but less promising than those obtained in Hodgkin's disease but acute lymphatic leukemia has failed to respond. Myelogenous leukemia apparently does not respond as well as the lymphocytic type but promising results have been obtained in polycythemia vera. The pain of multiple myeloma has been controlled but otherwise the nitrogen mustards have been without value in this condition. They have been used in inoperable bronchogenic carcinoma but the results are equivocal although some patients are apparently improved. Other types of malignancies in inoperable stages have been treated without appreciable success.

Before using the nitrogen mustards one must carefully consider the patient's condition and the possibilities of benefit since the nausea, vomiting, and anorexia are occasionally severe and their use may do more harm than good in a seriously ill patient.

LYMPHOCYTIC TYPE OF LYMPHOMA

The lymphocytic type of lymphoma occurs in a leukemic or circulating form (lymphocytic leukemia) and a nonleukemic or noncirculating form (aleukemic leukemia, pseudoleukemia, small cell lymphosarcoma). The lymphocytic type of lymphoma with leukemia has been discussed as lymphocytic leukemia.

The form of lymphocytic lymphoma which is not accompanied by any alterations in the peripheral blood presents a histologic picture in the lymph nodes which is identical to that of the leukemic type. There is no adequate explanation as to why the leukemic cells enter the circulation in one patient and not in another. The normal architecture of the lymph nodes is replaced by small or medium-sized lymphocytes giving the appearance of a sac filled with cells. These collections of cells occur not only in lymph nodes and spleen but wherever lymphoid tissue in any form is located.

Lymphadenopathy is generalized, and no group of glands is apt to escape involvement. The nodes are soft and elastic in consistency and do not usually become as hard as in the late stage of Hodgkin's disease. They tend to be more uniform in size, and enormous masses of lymph nodes do not ordinarily develop. Pressure symptoms are less frequently encountered than in Hodgkin's disease although they may occur in the mediastinum.

None of the clinical features are sufficiently distinctive to differentiate this from Hodgkin's disease so that one must resort to biopsy and histologic examination of a lymph node to establish the diagnosis. The leukemic variety is recognized by the characteristic changes in the peripheral blood.

The course of the leukemic and aleukemic types of lymphocytic lymphoma are similar. An acute, rapidly progressive form may occur in children and young adults whereas the chronic variety is more frequent in later adult life. When it makes its appearance in late adult life, the disease may be extremely chronic in its course, lasting for many years and producing few clinical manifestations.

The results of irradiation therapy are similar to those obtained in Hodgkin's disease. Nitrogen mustards may be of temporary benefit in the chronic forms but seem to have less effect than in Hodgkin's disease.

LYMPHOBLASTIC TYPE OF LYMPHOMA

The lymphoblastic type of lymphoma is also known as lymphoblastoma or large cell lymphosarcoma. The pathologic picture is similar to that of the lymphocytic type except that the cells which fill the lymph nodes are large immature lymphoblasts rather than mature lymphocytes. There is a greater tendency for the cells of the lymphoblastic type of lymphoma to invade and penetrate the capsule of the node. Since they are more immature cells, the growth and progress of the disease is apt to be more rapid.

There are no characteristic changes in the peripheral blood stream in the usual case although a progressive anemia occurs in conjunction with the disease. In a relatively few instances a leukemic blood picture develops which is indistinguishable from acute lymphocytic leukemia.

The lymphadenopathy is not distinctive. A final diagnosis must be made by histologic study of the node taken for biopsy.

Röntgen therapy and the nitrogen mustards are used as in Hodgkin's disease but the course is rapid and the outlook poor.

FOLLICULAR TYPE OF LYMPHOMA

The follicular type of lymphoma presents a clinical picture similar to the other lymphomas, but histologic examination of a lymph node reveals multiple nodules of various size which compress and distort the lymph spaces and sinuses. The nodules or follicles are round or oval bodies filled with lymphoid cells which are chiefly of the large lymphoblastic type. There is

some tendency for fusion of the follicles so that a picture simulating lymphoblastic lymphoma may develop.

The diagnosis rests upon microscopic examination of an excised node.

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AGRANULOCYTOSIS

SYNONYMS: GRANULOCYTOPENIA, AGRANULOCYTIC ANGINA, MALIGNANT NEUTROPENIA, pernicious leukopenia

Agranulocytosis is a disease characterized by a sudden and marked reduction in the number of granulocytic cells in the blood stream and, usually, by ulcerative lesions of the mucous surfaces

If agranulocytosis is to be recognized as a distinct clinical entity, it must be separated from those diseases and infections which have leukopenia as one of their manifestations. The cause of many cases of agranulocytosis is unknown so that the disease is subdivided into two groups, one idiopathic in origin and the other secondary to a recognized causative agent. By far the greatest number are of the secondary type, and a vast majority of these follow the use of certain chemicals or drugs. There are a number of drugs which cause a depression of all of the bone marrow elements, but Kracke was the first to demonstrate a selective action of small amounts of benzene on the myelocytic cells. Madison and Squier first called attention to the fact that amidopyrine was the causative agent in a majority of the patients having agranulocytosis. It was noted that this disease was particularly frequent in women, especially those who were in some way associated with physicians, such as doctors' wives, nurses, technicians, and others who had easy access to drug supplies. The investigations culminated in the observation that amidopyrine was frequently used by these women for relief of headache or the pain associated with menstruation and that the agranulocytic syndrome was prone to follow its use. Confirmatory evidence of the effect of amidopyrine on the leukocytes was obtained by giving a small dose of the drug to patients who had recovered from an attack of agranulocytosis. This caused a sharp drop in the neutrophil count. Neutropenia does not occur in all individuals using amidopyrine, it is necessary to presuppose a susceptibility or sensitivity to the drug in the affected persons. The development of agranulocytosis under these circumstances does not preclude the possibility that some preexisting defect in the bone marrow may be present.

In addition to amidopyrine itself there have been numerous compounds on the market, available to patients without a prescription from a physician, which contain amidopyrine combined with other drugs. These frequently bear a trade name which does not suggest the presence of the offending substance. They are as dangerous as amidopyrine itself since the disease may be produced by a very small amount of the drug in susceptible individuals. Amidopyrine and its compounds have undoubtedly been the cause of a large percentage of the cases of agranulocytosis.

Other drugs have been less frequently but no less definitely incriminated in the production of this disorder. Dinitrophenol, formerly used for reducing body weight, not only causes agranulocytosis but leads to the development of cataracts. Organic arsenical preparations used in antisyphilitic therapy, such as arsphenamine and neoarsphenamine, have caused agranulocytosis, but mapharsen has been used with safety in patients in whom arsphenamine has previously caused agranulocytosis. Agranulocytosis has resulted from the use of bismuth in antisyphilitic therapy, gold salts in the treatment of arthritis, and tridione.

Thiouracil, which is extensively used in the treatment of hyperthyroidism, causes leukopenia in a rather high percentage of the patients to whom it is administered, a neutropenia with a total leukocyte count of from 3000 to 4000 being not uncommon. In a much smaller group of patients, apparently about 1 per cent of those taking the drug, a severe agranulocytosis develops. The complication unfortunately does not always occur early in the course of treatment but may appear after the drug has been given over a period of many weeks so that each patient must have frequent leukocyte counts throughout the course of treatment. Propyl-thiouracil is reported to be less apt to cause agranulocytosis but it is not without danger.

The sulfonamide compounds such as sulfanilamide, sulfapyridine, sulfathiazole, sulfadiazine, and succinylsulfathiazole are prone to cause agranulocytosis. This usually develops after prolonged administration of the drug although cases are reported in which the agranulocytosis developed suddenly soon after treatment was started. Watkins found several cases in which a barbiturate was apparently the causative agent of agranulocytosis but this group of drugs are infrequent offenders. Many other drugs and chemicals are reported to have produced agranulocytosis in isolated instances and a complete list of all of these is impractical but it must be remembered that any preparation containing a benzene ring in its chemical structure is potentially dangerous to susceptible individuals and their leukocyte count must be carefully watched.

Although drugs and chemicals are the most common cause of agranulocytosis there are many cases in which these seem to be definitely excluded as an etiologic agent. The disease has been thought to be the direct result of an overwhelming infection in some cases but it is more probable that the infection gained a foothold as a result of the lowered protective mechanism of the body rather than being the actual cause of the agranulocytosis. In many instances the white cell count has been found to be low several days before evidences of infection develop. It appears, however, that many of the severe depressions in the leukocyte count follow the use of amidopyrine or a similar drug in patients whose leukopoietic tissue has already been damaged by a severe infection. Allergic and endocrine disturbances have been suggested as etiologic or predisposing factors but this theory has not been fully substantiated although some features of the attack suggest an allergic reaction. Antibodies have not, however, been demonstrated in susceptible individuals, and skin tests and scratch tests have been negative. Reznikoff believes that fatigue and menstruation are important predisposing factors.

Investigations on the effect of pteroylglutamic (folic) acid and urethane and its allied compounds on hematopoietic and embryonic tissues may lead to a better understanding of agranulocytosis. The suggestion has been made that it is a reaction due to competition for certain essential substances or an interference with the enzyme system of the tissues which leads to a deficiency of cell production. The rapidity with which the agranulocytosis develops in some instances is more suggestive of cell destruction than of an arrest in their development although the latter mechanism is highly probable in more slowly developing cases of leukopenia.

The disease is more common in women than in men and occurs most frequently in mid adult life. Most cases have been encountered during the fifth decade. The incidence of the disease is much lower since the drugs which are apt to cause it have been recognized and their use has been restricted.

Pathology

The pathologic changes in the bone marrow in agranulocytosis are variable. Some of the discrepancies in the findings may be explained by the examinations being made at different stages of the disease and some on differences in the methods used. An extreme degree of hypoplasia of the myelopoietic tissues has been encountered in some cases, in others there is aplasia of all the cellular elements. In still other instances the cellularity of the hematopoietic marrow has been found to be essentially normal or actually hyperplastic with myelocytes and myeloblasts present in unusually large

numbers. To explain these discrepancies it has been suggested that there is a maturation arrest which stops the growth and development of the granulocytes or produces degenerative changes in the cells before they migrate to the peripheral blood stream. This maturation arrest occurs at the myeloblast-myelocyte stage of development. Since they do not mature to the proper stage for liberation into the blood stream, the young cells accumulate in greater than normal numbers in those areas where they are being formed. This type of hyperplastic cellular marrow is encountered during the early stage of the disease and is found in those patients who die with acute fulminating agranulocytosis. There is a terminal or secondary destructive reaction with hypoplasia and decreased cellularity of the bone marrow in those cases which are of longer duration.

Necrotic ulcerations may be found on the mucous surfaces of the mouth, esophagus, intestine, or vagina. These ulcers are sharply outlined and are characterized by a very slight inflammatory reaction and an almost complete absence of granulocytic leukocytes in the area.

Clinical Features

Agranulocytosis occurs in an acute and a chronic form. The acute form is characterized by the manifestations of an acute fulminating infection. The temperature rises rapidly and is accompanied by chills, headache, muscle pains, malaise, and extreme prostration. Nausea and vomiting or other gastrointestinal symptoms may occur. An extremely sore throat is present in a majority of the patients and is associated with a diffuse inflammation of the pharynx in the early phase of the disease. This progresses to ulcerative and necrotic lesions of varying size which involve the tonsils, pharynx, gums, or buccal mucous membranes and, in some cases, extend to the larynx and esophagus. The ulcers are sharply demarcated and covered by a necrotic membrane. They are extremely painful in most instances and seriously interfere with or completely prevent the patient from swallowing. A firm brawny edema may be present in the surrounding tissues. Gangrene and sloughing of the necrotic areas may occur. The regional lymph nodes become enlarged. Similar ulcerations may develop on the mucous surfaces elsewhere, especially about the rectum, vulva, and vagina. Soreness or aching of the gums has been noted in some instances in the early stage of the disease, before the fever or ulcerative lesions have made their appearance. The leukopenia is present, however, at the time these symptoms appear. The acute onset is sometimes preceded by a minor illness, an injury, an operation, or some other disorder for which anudopyrine or other drugs were taken. The course may

be rapid with a persistently high fever, delirium, coma, and death within a few days of the onset. A high unremitting temperature is of particularly grave significance. Bronchopneumonia is a frequent complication and cause of death. Neither hemorrhagic manifestations nor purpura occur with this disease although a skin rash is commonly encountered.

The course is less severe in many patients, the febrile reaction being lower and the lesions of the mucous membranes less prominent or absent. The picture is still that of an infectious process with chills, fever, and prostration, but these symptoms are less severe and the outlook is more hopeful than in the acute type. Fatigue and extreme weakness are prominent features in the less fulminating cases, a fatigability which is out of proportion to the infection which is present and which is difficult to explain on the basis of leukopenia alone.

Splenomegaly is not present in acute agranulocytosis. The regional lymph nodes may become enlarged as a result of the ulcerative lesions of the mucous membranes, but there is no generalized lymphadenopathy. Jaundice occasionally occurs as a result of hepatitis, but the liver is seldom enlarged. The urine contains albumin and casts.

Hematologic Features

The most characteristic features of agranulocytosis are the marked reduction in the total leukocyte count and the disappearance of the granulocytes from the blood stream. These have been found to precede the acute febrile onset of the disease by several days so that by the time the symptoms become severe the characteristic blood picture is well established. In those cases which are secondary to amidopyrine or other drugs there is a variable interval between the drug's administration and the appearance of the leukopenia. Most patients require only a small amount of the drug to bring about the leukopenia, and the symptoms appear within a few days. In a few cases the leukopenia develops only after prolonged administration of the causative agent.

The total leukocyte count is usually below 2500 cells per cubic millimeter and frequently it is less than 1000. Jackson reports that nearly a third of the cases have less than 500 white blood cells. Examination of the blood smear reveals that the reduction in the total leukocyte count is due to a reduced number of neutrophils. The percentage of neutrophils may drop to 10 or 15 per cent. In the very severe cases these cells are almost completely absent. Most of the neutrophils which are found in the blood stream are old and degenerated forms although occasionally an immature cell may be seen

Eosinophils are also absent from the blood stream. A vast majority of the cells on the smear are lymphocytes. In the early stage of the disease and in less severe cases the actual number of lymphocytes will remain normal so that a relative lymphocytosis results. In the late and severe stages there is a reduction in the number of lymphocytes as well as neutrophils, as must obviously be true when the total leukocyte count is below 1000. It is apparent therefore, that although the neutrophils are primarily affected in this disease the lymphocytes may also be involved. The monocytes are not significantly affected as a rule although it has been stated that when they are present in unusually large numbers, it is a hopeful prognostic sign.

When improvement begins the leukocyte count increases rapidly, the number and percentage of neutrophils rise, and there is a nuclear shift to the left signifying the appearance of young granulocytic cells in the blood stream. Myelocytes and metamyelocytes are frequently encountered at the onset of a remission.

There is no more change in the hemoglobin and erythrocyte levels than occurs with any acute infection, and anemia is not a significant part of the picture of agranulocytosis. The platelets are not affected so that hemorrhagic episodes do not appear.

Sternal puncture reveals a variable picture as indicated in the discussion of the pathology of the disease. The variations are probably explainable on the stage of the disease in which the examination is made and the severity of the particular case. In some instances there is a hypercellularity with many immature granulocytic cells. This may represent a maturation arrest or it may indicate beginning recovery. In other cases there has been aplasia of the granulocytic series of cells and this is the common finding in the severe forms of the disease. Sternal aspiration is of greatest value in excluding aleukemic leukemia and aplastic anemia in the differential diagnosis.

Diagnosis

The diagnosis of agranulocytosis is based upon the characteristic blood findings. These may or may not be associated with necrotic ulcerations of the mucous membranes. A history of having used one of the incriminated drugs is of value in establishing the etiology of the disorder but such a history cannot be obtained in many instances. Every effort should be made to find the responsible agent.

The disease must be differentiated from those infectious diseases which are commonly accompanied by leukopenia and a relative lymphocytosis such as

influenza, typhoid fever, typhus, measles, and the early stage of infectious mononucleosis. It must also be differentiated from those blood dyscrasias which cause leukopenia, such as pernicious anemia, Banti's syndrome, and primary splenic neutropenia. None of these causes such a profound change in the neutrophil count as occurs with agranulocytosis.

Greater difficulty is encountered in differentiating this disorder from aleukemic leukemia and aplastic anemia. Aleukemic leukemia may occasionally produce a marked leukopenia, and there are immature cells in the blood stream, enlargement of the spleen or lymph nodes, progressive anemia, and thrombopenia. It is rare for an acute aleukemic leukemia not to show some immature cells in the blood stream, but if the diagnosis is in doubt, a sternal puncture and examination of the aspirated marrow may settle the problem.

In aplastic anemia, which may cause difficulty in the differential diagnosis, the erythrocytes and platelets are lowered as well as the leukocytes. Severe hemorrhagic manifestations are common in aplastic anemia, and the anemia is more profound.

Prognosis

The mortality in agranulocytosis formerly ranged from 70 to 85 per cent. Recognition of the causative drugs, their restricted use and immediate withdrawal, together with the use of chemotherapeutic and antibiotic preparations to curb the secondary infection have greatly improved the outlook. The mortality rate also appeared to be lowered by the use of pentnucleotide although this is doubted by some. In spite of the protective measures now available it is still a dangerous disease. The prognosis is poor if the leukocyte count is below 1000 and is worse in older patients than in younger ones. Death occurs as a result of infection, commonly with sepsis or bronchopneumonia.

When recovery ensues there is a rise in the leukocyte count with an increase of the granulocytes in the peripheral blood. Varying numbers of immature forms appear in the blood stream. The increase in the leukocyte count may be rapid and a leukocytosis frequently follows, commonly reaching 12,000 to 15,000.

Treatment

The first step in the treatment of agranulocytosis is to discover and remove the etiologic agent. There are numerous proprietary preparations for the relief of pain which contain amidopyrine or pyramidon, and the patient should be carefully questioned regarding the use of any drug of this type.

The offending drug, or any drug which is closely related from a chemical standpoint, should not be given. This holds true even though no history of its use is obtained.

Since infection is the greatest danger and the principal cause of death in agranulocytosis every possible effort should be directed toward preventing and treating infection. Penicillin has been of the greatest value in this regard and should be started immediately in adequate dosage and continued until the infection has subsided or until the danger of infection has passed. It is given in dosages of 50,000 to 100,000 units every three hours, or in larger amounts if necessary. The use of this antibiotic has reduced the mortality of agranulocytosis immeasurably. The sulfonamides may be used in the same manner but their propensity for producing agranulocytosis limits their usefulness in the treatment of this condition. They may be used in treating those patients in whom the agranulocytosis was not produced by their use, and have on occasion been used in patients in whom they appeared to be the causative agent. Their use is seldom justified, however, in the presence of agranulocytosis. It should be realized that penicillin is used for the treatment and prevention of infection until the normal protective mechanism of the body has recovered. It has no effect on the bone marrow and in no way stimulates the formation and development of granulocytes. General supportive measures should be used. If there is difficulty in swallowing because of infection or necrotic lesions it will be necessary to administer fluids parenterally and in every case a large fluid intake should be maintained.

Transfusions are of value as a supportive measure although they have no specific effect on the agranulocytosis. They are best given in relatively small amounts rather than as single large transfusions. From 200 to 250 cc. of blood at daily intervals in the seriously ill patient is advisable.

Pentnucleotide appears to be the most beneficial therapeutic agent although some observers question its value and do not believe that it is of any benefit. Jackson and Tighe found that the mortality was greatly reduced by its use. In our own experience it has apparently been effective. Pentnucleotide is administered intramuscularly in 10 cc doses and is given four times a day until the leukocyte count begins to rise and then once or twice a day until the count has returned to normal. Definite evidences of improvement will usually appear on the fourth or fifth day of its administration. If no beneficial results are apparent by the tenth day of its use, it will not be effective and may be discontinued. Rather alarming reactions, shortness of breath or a sense of substernal constriction, are encountered rather frequently after its

administration. The necessity for a preparation of this type has been greatly reduced by the use of penicillin.

Liver extract by intramuscular injection has been advocated but it is doubtful if it has any significant effect. Roentgen ray therapy in the form of small doses designed to stimulate the bone marrow has also been used. Its apparent beneficial effect seems to be due to a hastening of the maturation of the leukocytes rather than to an actual stimulation of the bone marrow. It has more of a degenerative than a stimulating effect and has been largely discontinued. Nonspecific therapy in the form of foreign protein injections has also proved to be worthless.

Adenine sulfate and bone marrow extract have been used in the treatment of agranulocytosis, but the results are equivocal. Both of these preparations should be regarded as being in the experimental stage.

Pteroylglutamic (folic) acid has been advocated in the treatment of agranulocytosis but there is as yet no conclusive evidence of its value in this condition. Biotin and pyridoxine have a similar status.

PRIMARY SPLENIC NEUTROPENIA

Primary splenic neutropenia is a syndrome described by Wiseman and Doan in which a profound neutropenia is the most outstanding feature. It is thought to be closely allied to thrombopenic purpura and hemolytic icterus although the reduction in the number of thrombocytes and erythrocytes is far less than the reduction in the number of neutrophils. Hemorrhagic features do not occur and anemia, when present, is of only moderate severity. The course of the disease may be acute or chronic and the clinical manifestations may include fever, ulcerations of the oral mucous membranes, chronic infections, abscesses, and other manifestations which reflect the patient's increased susceptibility to infection. Chronic ulcerations of the skin over the shins have been observed.

The spleen is enlarged and pain in the splenic area has been reported. The bone marrow shows myeloid hyperplasia but not of a leukemic type. There are no demonstrable evidences of liver damage as in Banti's syndrome.

The microscopic appearance of the spleen has been variable. Wiseman and Doan reported evidences of increased phagocytosis of neutrophils and erythrocytes by the macrophages of the spleen and hyperplasia of the reticulo-endothelial elements, but these pathologic features have not been found consistently by other observers.

The pathogenesis of this form of neutropenia has been assumed to be a too

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PRIMARY SPLENIC NEUTROPENIA

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rapid removal and destruction of the neutrophils by the spleen and this is borne out by the evidences of increased phagocytosis within this organ and the myeloid hyperplasia of the bone marrow. Other observers have hypothesized that a substance formed and liberated by the spleen suppresses the development of the neutrophils in the bone marrow. The condition must, at the present time, be considered as a clinical syndrome rather than as a pathologic entity but its recognition is an indication for splenectomy.

Removal of the spleen in primary splenic neutropenia leads to complete and permanent recovery from both the clinical and laboratory standpoint.

Primary Splenic Panhematopenia

Doan has also reported cases in which there is indiscriminate destruction of circulating elements of all types (erythrocytes, leukocytes, and platelets) by the spleen. There is a compensatory hyperplasia of all cellular elements of the bone marrow but despite the increased regeneration of cells in the marrow they are depleted in the peripheral blood. This may occur as a primary and congenital dysfunction of the spleen and removal of the organ under these circumstances is curative. The syndrome may also occur secondary to other types of splenic disease.

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INFECTIOUS MONONUCLEOSIS AND INFECTIOUS LYMPHOCYTOSIS

INFECTIOUS MONONUCLEOSIS IS AN ACUTE INFECTIOUS DISEASE OF UNKNOWN cause characterized by fever, lymphadenopathy, splenomegaly, and the presence of many abnormal lymphocytes in the blood stream. The term *infectious mononucleosis* is used synonymously with *glandular fever*, a name given to the disease by Pfeiffer in 1889. There has been some discussion as to whether these terms refer to the same clinical entity, but the present consensus is that the diseases are identical.

Infectious mononucleosis is widespread throughout the world and occurs in sporadic, endemic, and epidemic forms. It is possible that many of the sporadic cases represent the more severe instances of a small epidemic in which many of the cases are so mild as to escape notice. The disease is most frequently encountered in children but is common in young adults as is exemplified in the epidemics encountered in the Army camps during World War II. No age is exempt and 10 per cent of the cases in one epidemic were between 45 and 65 years of age. Many cases have been reported from hospital clinics so that the apparent incidence in young adults, such as medical students, nurses, and the hospital resident staff, has been unduly high. Recent reports indicate that the disease is not uncommon in Negroes.

Etiology

The cause of infectious mononucleosis is unknown but evidence that it is a specific virus infection has been steadily accumulating. Absolute proof, however, is still lacking and attempts to transmit the disease to laboratory animals and to human subjects have not been wholly successful in reproducing all manifestations of the disease. Many different organisms have been suspected of being the causative agent and several have been isolated from the blood stream of patients with the disease but adequate proof has never been forthcoming to incriminate definitely any one of these.

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tons have appeared. Soreness of the throat appears at varying intervals after the premonitory symptoms and may be a mild discomfort or extremely painful with a severe inflammatory reaction of the pharynx and tonsils. A patchy or a diffuse membrane may be present, but ulcerations are not common. The glands are enlarged out of proportion to the severity of the pharyngeal involvement in most cases. This feature frequently calls attention to the possibility of infectious mononucleosis rather than a simple pharyngitis as a diagnosis. In a few cases the glandular involvement is minimal or absent. Many such cases undoubtedly escape recognition, and a diagnosis of simple pharyngitis or tonsillitis remains unquestioned. Fever is present throughout the acute phase of the illness and may reach 104 or 105 F. Although the severity varies, the disease usually runs its course in two or three weeks and spontaneously subsides. Fatigue and lassitude may persist, and the lymphadenopathy may remain for months after the acute febrile episode has subsided.

The *glandular or Pfeiffer type* of infectious mononucleosis may occur sporadically but is more frequently epidemic. It is the type most commonly encountered in children. It differs from the pharyngeal variety in that the throat symptoms are mild or absent and a generalized lymphadenopathy is the predominant feature. Enlargement of the mediastinal nodes may cause irritation and cough. Retroperitoneal and mesenteric lymphadenopathy may produce abdominal pain and gastrointestinal symptoms. The course of the disease is similar to that of the pharyngeal type—rather acute and rapid—so that recovery is usually complete within two or three weeks.

The *febrile type*, described by Tidy, has as its predominant feature a febrile course with headache, malaise, and generalized muscular aching. Soreness of the throat is rare, and glandular enlargement is minimal and does not appear until about the third week of illness. After several days of rather high fever the temperature subsides, but recurrences are common and the course of the disease may be prolonged.

Clinical Features

The accompanying table shows the frequency with which the various symptoms and findings were encountered in an epidemic of infectious mononucleosis observed by Baldrige, Rohner, and Hansmann.

Symptoms	Percentage	Physical Observations	Percentage
Headache	70	Fever	100
Malaise	70	Enlarged glands	100
Sore throat	68	Post cervical	88
Tender glands	60	Axillary	86

Although infectious mononucleosis is a contagious disease, the degree of contagiousness is so low that quarantine and strict isolation of sporadic cases are unnecessary. Some degree of segregation is advisable during the febrile period, however. The contagiousness is much greater during epidemics than with sporadic cases.

Pathology

The lymph nodes removed for histologic examination are soft and spongy in consistency with the capsule distended and white. The nodes appear gray and granular on cut section, and the normal architecture is distorted by lymphoid hyperplasia, which compresses the sinuses and distends the capsule. Some germinal centers are entirely replaced by lymphoid hyperplasia, the cells containing numerous mitotic figures. There are many lymphoid cells free in the lymph sinuses. The fibrous reticulum of the node is not increased, and there are no areas of necrosis.

The bone marrow does not show the lymphocytic infiltration and replacement which characterize the marrow in lymphocytic leukemia but rather a myeloid hyperplasia of the type seen in other infections.

Clinical Types

The incubation period of infectious mononucleosis is apparently variable, usually ranging from ten to fourteen days. The clinical manifestations likewise are extremely variable and it has been stated that it is second only to syphilis in its ability to mimic other conditions. Several clinical types are commonly described. These subdivisions are based upon the predominant manifestations but there is a great deal of overlapping. The onset may be insidious or acute, the sporadic cases frequently having a prodromal period of several days or a week with anorexia, malaise, fatigability, and headache. In other cases the onset is manifest by fever, chills, sore throat, and more severe prostration. Varying degrees of enlargement of the lymph nodes and spleen are usually encountered although these features may not appear until later in the course of the illness.

The pharyngeal type of infectious mononucleosis is one of the most frequent. It is more common in young adults than in children and tends to occur sporadically in the spring and fall rather than in epidemic form. The onset is usually insidious and is characterized by a few days of malaise, lassitude, headache, generalized muscle soreness, and chilly sensations, following which there are fever, chills, and night sweats. The lymph nodes become enlarged and the spleen becomes palpable a few days or a week after the other symp-

is common but nausea and vomiting are relatively infrequent. Diarrhea is not a common symptom and is encountered less frequently than is constipation.

The mediastinal nodes are involved but do not become extremely large. A widening of the mediastinum may be detected on roentgenologic examination in some instances but whereas coughing is a frequent symptom there is no correlation between the presence of cough and increased mediastinal shadows. Pulmonary complications occasionally arise with cough, expectoration, pain in the chest, and the presence of sibilant rales. Roentgenologic examination occasionally shows a haziness or mottling indistinguishable from that of atypical pneumonia. It clears rapidly.

Lymphadenopathy may appear in one area, disappear as the acute manifestations subside, and reappear in another area with recurrence of a febrile course. In most instances all of the nodes become enlarged at about the same time although in some the lymphadenopathy spreads from one area to another. The lymphadenopathy usually regresses as the acute manifestations disappear, but it is not uncommon for a slight enlargement of the nodes to persist for months after all other clinical manifestations have subsided.

The spleen is palpable in about 75 per cent of the patients. The area of splenic dullness is increased in many others in whom the organ is not large enough to be felt. The enlargement is not extreme, and the edge seldom descends more than 3 or 4 cm below the costal margin. In an occasional patient the spleen may be considerably larger. It is frequently tender, and complaints of a dull ache, soreness, or of sharp pleurisy-like pains in the splenic area are not infrequent. The size of the spleen slowly diminishes as the acute stage of the disease subsides, but splenomegaly may persist for months after the acute attack. The liver is occasionally enlarged, usually to only a slight degree although it may extend a handbreadth below the right costal margin. It may be slightly tender.

Various types of skin lesions may appear during the course of the disease, the frequency varying in different epidemics from 4 to 18.5 per cent. In our experience they have not been common and occurred in only a small percentage of the cases. They may be maculopapular, urticaria-like lesions, or a generalized rubelliform eruption. Petechiae occasionally occur in the skin and mucous membranes.

There are no severe hemorrhagic manifestations, but slight epistaxis and bleeding gums are occasionally encountered. Hematuria may be present as a result of acute focal nephritis, but diffuse glomerular nephritis does not occur. A granular conjunctivitis may develop during the course of the disease. Edema of the eyelids is sometimes noted. Coughing may result from irrita-

Symptoms	Percentage	Physical Observations	Percentage
Backache	54	Subangular	64
Chilliness	44	Submaxillary	52
Anorexia	38	Inguinal	50
Sweating	34	Epitrochlear	32
Weakness	32	Submental	10
Cough	30	Tender glands	76
Dizziness	26	Enlarged spleen	48
Sore bleeding gums	26	Enlarged tonsils	44
Nausea	24	Membranous angina	22
Stiff neck	24	Enlarged liver	16
Abdominal pain	16	Throat injected	58
Vomiting	12	Peritonsillar abscess	12
Photophobia	10	Abdominal tenderness	4
Aphthous sore mouth	10		
Pleurisy	8		
Loss of weight	6		
Fainting	6		
Constipation	4		
Diarrhea	2		

Enlargement of lymph nodes is the most characteristic and constant feature of this disease. Although an occasional case is encountered without apparent lymphadenopathy, this is rare. The enlargement is usually generalized and involves all of the superficial nodes, but sometimes only those of the cervical region will be obviously affected. The cervical nodes rarely escape, particularly those of the anterior chain. The size of the nodes will vary from one patient to another as well as in various locations in the same patient. They do not reach the extremely large size of those encountered in Hodgkin's disease. Ordinarily the nodes are about 1 cm in diameter with an occasional one, particularly beneath the mandible or in the axilla, being 3 or 4 cm. in diameter. They are rather soft and rubbery in consistency, they are not attached to the surrounding tissue so that they remain freely movable, and they do not involve the overlying skin. The discomfort produced by the glands is variable. In many instances the patient is completely unaware of their presence since there has been no pain or tenderness. In other cases the nodes are tender and cause pain and stiffness of the neck. Suppuration does not occur except in rare instances. Lymphatic drainage from certain areas may be blocked, which may lead to edema.

The mesenteric nodes are seldom sufficiently enlarged to be detected on physical examination but they are undoubtedly involved more frequently than is generally supposed. Abdominal pain is a common symptom, sometimes being severe enough to simulate appendicitis or regional ileitis, and the mesenteric lymphadenopathy may contribute to its production. Anorexia

no greater anemia than would be found with any acute infection. The absence of anemia is important in distinguishing this disease from leukemia. The blood platelets are unaffected, and although thrombopenia has occasionally been encountered, it is a rare complication.

The total leukocyte count is variable, usually being from 10,000 to 20,000 by the time the patient consults a physician. Occasionally it is elevated to 50,000 or 60,000, but this is unusual. Leukopenia is a frequent or perhaps a

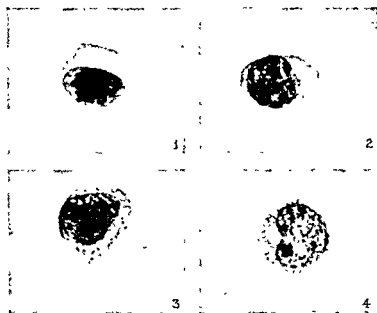


FIG. 73. Photomicrographs of abnormal lymphocytes from the blood of patients with infectious mononucleosis. Vacuolation of the cytoplasm is particularly striking in the cell on the lower right.

constant feature of the very early stage of the disease. Leukocyte counts of 3000 to 4000 at this time are not infrequent.

A neutrophilic leukocytosis occurs early in the course of the disease but soon gives way to a lymphocytosis. Lymphocytes usually comprise 50 to 90 per cent of the white blood cells within a few days of the onset of the disease although they may not become prominent until the second week.

The "abnormal lymphocytes" which appear in the blood stream are the most characteristic hematologic feature (Fig 73). These cells vary in their structure and appearance but are mature forms of the lymphocytic series and must be differentiated from immature lymphocytes, which are found in leukemia. Most of the lymphocytes encountered on the smear are ab-

tion caused by enlargement of mediastinal lymph nodes but is more frequently due to an extension of the inflammatory process to the larynx, trachea, and bronchi.

A positive Wassermann reaction is often encountered in infectious mononucleosis. Tidy found this to occur in about half of the cases, the reaction becoming positive during the second week of the illness, persisting for several weeks, and then becoming negative. In our experience such a reaction has been less frequent.

Jaundice occasionally occurs as a complication of infectious mononucleosis as a result of diffuse hepatitis which may be accompanied by an obstruction of the bile ducts and a derangement of liver function. The liver may be enlarged and tender, occasionally extending four or five centimeters below the costal margin. Wechsler, Rosenblum, and Sills state that the clinical picture of infectious mononucleosis complicated by hepatitis was indistinguishable from the ordinary case of infectious hepatitis except that the icterus cleared more rapidly and the gastrointestinal symptoms were milder. It is doubtful if jaundice results from pressure on the bile ducts by enlarged nodes as has frequently been suggested. When hepatitis occurs in conjunction with small shotty lymph nodes and a positive Wassermann reaction it is extremely suggestive of a diagnosis of early syphilis.

Meningitis and meningo-encephalitis have been observed during the course of infectious mononucleosis although their incidence is extremely low. Headache is a particularly troublesome feature when these complications occur and is associated with other evidences of meningeal irritation or cerebral involvement such as mental confusion, irritability, difficulty with speech, lethargy, and cranial nerve palsies. The spinal fluid shows an elevated cell count ranging from 25 to 300 cells per cubic millimeter, most of which are lymphocytes.

Cardiac complications have been mentioned by Bernstein and by Wechsler but are apparently uncommon. Abnormal rhythms as well as S-T segment changes and low voltage of the QRS complexes in the electrocardiograms were encountered during the course of the disease but definite evidence of permanent damage to the heart is lacking.

Hematologic Features

The most striking features of the disease are to be found upon examination of the blood. The diagnosis can be established with reasonable certainty in a majority of the patients by this means. The erythrocytes and hemoglobin level are not significantly affected by infectious mononucleosis, and there is



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normal in their structural characteristics, only a few normal lymphocytes being present. An occasional abnormal lymphocyte may occur in other diseases which are characterized by lymphadenopathy, but there is none in which they are so prominent and form such a large percentage of the lymphocytes in the blood stream. Downey and McKinlay divided these abnormal lymphocytes into three types, emphasizing the fact that they must be recognized primarily by the inner structure of the nucleus rather than by the size or configuration of either the nucleus or the cell itself.

The size of these abnormal lymphocytes varies from a cell which is but slightly larger than a normal mature lymphocyte to one which approaches or exceeds the size of a monocyte. The nucleus may be round or oval but is frequently irregular in outline and appears to be lobulated. The chromatin is arranged in coarser strands than is normal for the lymphocyte and forms a network containing ill defined masses of chromatin material. In some cells these blocks of chromatin are grouped at the periphery of the nucleus so that its appearance is suggestive of a plasma cell. In others there is a dense ring of chromatin a short distance inside the outer margin of the nucleus leaving the outer ring and central portion of the nucleus lightly stained. The chromatin of some cells is arranged in a close network of fine strands giving an even diffuse staining reaction which resembles that of an immature cell. Vacuoles and fenestration of the nucleus are frequent. Osgood has placed considerable stress on this feature in the diagnosis of infectious mononucleosis.

The nucleus of the abnormal lymphocyte may almost completely fill the cell. More frequently it is eccentrically placed and is surrounded by cytoplasm which is more abundant than normal. The cytoplasm stains more

PLATE VIII.

Abnormal types of lymphocytes as encountered in the blood of patients with infectious mononucleosis. Cells 1 to 11 are mature but abnormal forms differing from the normal in their cytoplasmic granulation, vacuolation, or depth of staining and in the chromatin structure of their nuclei. Cells 12 to 14 are immature cells from a case of acute lymphocytic leukemia for comparison. (Heck, Infectious mononucleosis. In Downey's Handbook of Hematology Paul B Hoeber, Inc.)

deeply and is more basophilic than normal for the lymphocyte and in some instances approaches the deep basophilia characteristic of the plasma cell. In place of the clear hyaline blue cytoplasm of the normal lymphocyte is a darker, mottled, and blotchy cytoplasm. The cytoplasm at the periphery of the cell frequently stains more deeply than that which is close to the nucleus. If the nucleus is indented, the cytoplasm in the bend of the nucleus is particularly light staining.

Azure granules are found in most but not all of these abnormal cells, and the number of granules in each cell is usually greater than normal. Many of the granules are larger and more prominent than the usual azure granules of the lymphocyte. Vacuolation of the cytoplasm is common, some cells have only one vacuole whereas in others the entire cytoplasm is studded or there is a ring of vacuoles just inside the cell membrane. The cells vary greatly in their size and structure but they are mature cells. Only occasionally do immature lymphocytes gain access to the blood stream in this disease.

Although varying numbers of normal lymphocytes will be present on the smear, ordinarily they form a relatively small percentage. The abnormal lymphocyte is not, however, pathognomonic of infectious mononucleosis.

Monocytes are present in normal or slightly increased numbers in infectious mononucleosis and may show an increased granularity of their cytoplasm. This monocytosis may persist during the convalescent period.

The percentage of abnormal lymphocytes decreases after the clinical manifestations of the disease disappear. Their disappearance from the blood stream lags behind the clinical improvement, and in many instances they persist for months after the other features of the disease have subsided.

HETEROPHILE ANTIBODY TEST FOR INFECTIOUS MONONUCLEOSIS

In 1932 Paul and Bunnell found that highly diluted blood serum from patients with infectious mononucleosis would agglutinate and hemolyze sheep's erythrocytes. This observation has been amply confirmed by other investigators and is now recognized as a reliable method for the serologic diagnosis of this disease. A low dilution of blood serum from a majority of normal persons is also capable of causing agglutination and hemolysis of sheep's erythrocytes. The agglutinins and hemolysins which produce this reaction are known as heterophile antibodies. The number of antibodies is increased after injections of horse serum, especially if serum sickness results. The heterophile antibodies which are present in normal serum and which develop after injections of horse serum can be removed from the serum by absorption with guinea pig kidney tissue whereas the antibodies which ap-

phocytes in the blood stream, and a positive heterophile antibody test. The clinical manifestations are extremely variable and may simulate a great many diseases so that numerous problems arise in the differential diagnosis.

Many cases of infectious mononucleosis are suggestive of lymphocytic leukemia because of the generalized lymphadenopathy, splenomegaly, fever, prostration, and preponderance of large cells of the lymphocytic series in the blood stream. The differentiation can be made from the blood smear in most instances, immature lymphocytes being present in those patients with leukemia whereas in infectious mononucleosis the lymphocytes are large and abnormal but mature cells. Although anemia is present in leukemia, in infectious mononucleosis the hemoglobin, erythrocytes, and platelets are not significantly affected. The heterophile antibody test is also of value in distinguishing between these diseases.

Leukocyte and differential counts are not made on many patients with acute pharyngitis. Undoubtedly some of these patients have unrecognized infectious mononucleosis. A careful differential count should distinguish between the two diseases.

Infectious hepatitis may be simulated in those instances of infectious mononucleosis which are characterized by fever, gastrointestinal symptoms, and jaundice. Generalized lymphadenopathy does not occur with infectious hepatitis and a careful examination of the blood smear should make the differentiation. The disease may also be confused with brucellosis.

Because of the falsely positive Wassermann reaction that occasionally occurs during the active stage of infectious mononucleosis, the disease may be confused with early syphilis, particularly if the involved glands are small and firm. The Wassermann reaction in infectious mononucleosis usually remains positive for two or three weeks and spontaneously becomes negative.

Although the distinctive blood picture and the heterophile antibody test serve to establish the diagnosis of infectious mononucleosis, there are many diseases which it may simulate under certain conditions. In addition to those already mentioned it may at times resemble typhoid fever, miliary tuberculosis, septicemia, influenza, tularemia, and Hodgkin's disease. When abdominal symptoms are a prominent feature, appendicitis or regional ileitis may be simulated.

Course and Prognosis

The course of the disease is extremely variable. In a majority of the patients it runs from one to three weeks with complete recovery occurring spontaneously. In other instances the malaise and lassitude may persist for

pear in the serum of patients with infectious mononucleosis are not absorbed to the same extent by this means.

In performing the heterophile antibody test varying dilutions of the suspected serum are added to a 2 per cent suspension of washed sheep's erythrocytes, and the highest dilution which causes agglutination of the erythrocytes is noted. Agglutination of the erythrocytes in a serum titer of 1:64 is suggestive. When it occurs in a titer of 1:224 or over, it may be considered as presumptive evidence of infectious mononucleosis provided the patient does not have serum sickness at the time the test is made. This procedure is known as the "presumptive test." If any doubt as to the diagnosis remains, the "differential" test should be employed.

The differential test is based on the fact that the heterophile antibodies of infectious mononucleosis are not completely absorbed from the serum when it is exposed to a suspension of guinea pig kidney tissue. In performing this test 0.1 cc. of the suspected serum, which has been inactivated, is mixed with 0.5 cc. of a 20% suspension of boiled guinea pig tissue. The mixture is centrifuged after standing for an hour. The supernatant fluid is added to the suspension of sheep cells in varying dilutions just as in the presumptive test. The guinea pig tissue removes some of the antibodies, but agglutination should occur in a titer which is at least one-fourth as high as that which caused agglutination in the presumptive test; i.e., not over three-fourths of the agglutinins are removed. If the presumptive test showed agglutination in a titer of 1:224, there should be agglutination in the differential test in a titer of 1:56 or above if infectious mononucleosis is present. If more than 90 per cent of the agglutinins are removed by absorption, the diagnosis of infectious mononucleosis is doubtful.

The heterophile antibody test becomes positive rather early in the disease in a majority of the patients, probably from 7 to 10 days after the onset. In some cases a positive reaction will not be obtained until as many as 21 days of the illness have passed. The length of time that these antibodies persist in the blood serum varies, but they are present long after the acute manifestations have subsided. They have been found to persist for as long as 296 days (average 119) after the acute phase of the disease has passed. The heterophile antibody test does not become positive in a small, but as yet undetermined, number of cases of infectious mononucleosis.

Diagnosis

The diagnosis of infectious mononucleosis is based upon the febrile course, generalized lymphadenopathy, splenomegaly, appearance of abnormal lym-

nantly mature small lymphocytes. It is apparently unrelated to infectious mononucleosis although the lymphocytic cells are predominantly affected in both diseases. It is primarily a disease of childhood although cases in young adults have also been reported. Evidences of an upper respiratory tract infection have been common and a skin rash, usually maculopapular in type, is frequently present. The clinical manifestations are usually mild and of short duration but may be entirely absent. In some instances fever, vomiting, and abdominal pain have been prominent, suggesting the need of surgical intervention. Irritability, headache and pain in various parts of the body have been described. Lymphadenopathy and splenomegaly have been described in some cases but are usually absent.

A leukocytosis with a preponderance of mature lymphocytes is the outstanding hematologic feature, the total leucocyte count reaching 50,000 or over in some instances with from 80 to 90 per cent of the cells being lymphocytes. A slight eosinophilia has been reported in some cases. There is no anemia and no thrombocytopenia. Abnormal lymphocytes of the type found in infectious mononucleosis are absent. Aspiration of sternal marrow shows an abnormally high percentage of small adult lymphocytes without evidences of a leukemic infiltration of immature forms.

Although the clinical manifestations are self-limited and of short duration the lymphocytosis of the blood and sternal marrow may persist for several weeks. The heterophile antibody test is negative. An increased number of lymphocytes has been found in the spinal fluid.

The disease must be differentiated from lymphatic leukemia, infectious mononucleosis, and pertussis by the hematologic, serologic, and bacteriologic methods.

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several weeks after the febrile reaction has subsided. The disorder may be so mild throughout its entire course that the patient has few complaints and the disease would not be suspected if blood examinations were not made on exposed individuals during an epidemic. In other cases there is a stormy course with a high fever and marked prostration.

The enlarged nodes and splenomegaly usually subside within a few weeks but occasionally persist for months or years. The abnormal lymphocytes may also persist in the blood stream for months after the acute episode subsides.

Complete and spontaneous recovery is the rule, and the disease itself is not fatal. Complications are extremely rare, but suppuration of the glands and bronchopneumonia have been observed. Spontaneous rupture of the spleen during the course of the illness has occurred in several cases. Recurrences and relapses of the disease are common. The prolonged convalescent period encountered in many instances, even in the mild forms of the disease, is a discouraging feature to many patients.

Treatment

Rest in bed, adequate fluids, and other forms of symptomatic treatment during the acute febrile period are all that is necessary in the way of therapy in a vast majority of patients. Specific convalescent serum has been used and appears to shorten the febrile period of the disease and to produce marked subjective improvement. Its use is justified only in severe cases. The serum should be obtained from a patient who has recently recovered from the disease, being drawn one or two weeks after the fever has subsided. From 50 to 300 cc. are administered intravenously. The sulfonamide drugs have been used but with no apparent effect on the course of the disease. Roentgen ray therapy to the lymph nodes is of no benefit.

Hot or cold compresses may mitigate the soreness and pain in the lymph nodes, and aspirin or codeine relieve the headache and generalized muscular aching. Blood transfusions may be given for subjective relief when the course is protracted. It is seldom necessary to resort to this procedure.

ACUTE INFECTIOUS LYMPHOCYTOSIS

Acute infectious lymphocytosis has been described by Smith, and subsequently by a number of others, as an acute infectious and contagious disease characterized by an absolute lymphocytosis in which the cells are predomi-

nantly mature small lymphocytes. It is apparently unrelated to infectious mononucleosis although the lymphocytic cells are predominantly affected in both diseases. It is primarily a disease of childhood although cases in young adults have also been reported. Evidences of an upper respiratory tract infection have been common and a skin rash, usually maculopapular in type, is frequently present. The clinical manifestations are usually mild and of short duration but may be entirely absent. In some instances fever, vomiting, and abdominal pain have been prominent, suggesting the need of surgical intervention. Irritability, headache and pain in various parts of the body have been described. Lymphadenopathy and splenomegaly have been described in some cases but are usually absent.

A leukocytosis with a preponderance of mature lymphocytes is the outstanding hematologic feature, the total leucocyte count reaching 50,000 or over in some instances with from 80 to 90 per cent of the cells being lymphocytes. A slight eosinophilia has been reported in some cases. There is no anemia and no thrombocytopenia. Abnormal lymphocytes of the type found in infectious mononucleosis are absent. Aspiration of sternal marrow shows an abnormally high percentage of small adult lymphocytes without evidences of a leukemic infiltration of immature forms.

Although the clinical manifestations are self-limited and of short duration the lymphocytosis of the blood and sternal marrow may persist for several weeks. The heterophile antibody test is negative. An increased number of lymphocytes has been found in the spinal fluid.

The disease must be differentiated from lymphatic leukemia, infectious mononucleosis, and pertussis by the hematologic, serologic, and bacteriologic methods.

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MULTIPLE MYELOMA AND LIPOID DYSTROPHIES

MULTIPLE MYELOMA

MULTIPLE MYELOMA IS A NEOPLASTIC DISEASE CHARACTERIZED BY MULTIPLE tumor masses originating in the bone marrow from one of the various types of cells which are normally present in the hematopoietic portion of the marrow. These tumors apparently arise from any of the marrow cells and they have been classified accordingly. The plasma cell type—plasmacytoma—has been by far the most common but there is a wide diversity of opinion as to the origin and nature of these plasma cells. It is probable that they arise from reticulo-endothelial cells but whether or not they represent an abnormal form of lymphocyte or an entirely separate series of cells is open to question. There is a growing tendency to speak of them as myeloma cells rather than as plasma cells. In addition to this type of myeloma there have been described myeloblastomas, erythroblastomas, and lymphocytomas, but these are extremely rare and the existence of these latter types has been questioned by some observers who feel that all are of the plasma or myeloma cell type.

The disease is characterized by widespread lesions of the skeletal system with the bones containing hematopoietic marrow being most frequently and most extensively involved. Although the lesions may occur in any bone they are more common in the skull, ribs, pelvis, and vertebra. The lesions are usually multiple by the time the disease is recognized, and it appears to originate in multiple foci rather than to spread or metastasize from one original area. In many instances only a single lesion is found on roentgenologic examination, but subsequent histologic study of the bones reveals multiple and extensive involvement. A few cases of apparently solitary myeloma have been recorded.

The disease is most common in the sixth decade of life, and males outnumber females about 3. 1.

Pathology

The lesions in the bones are grayish-red in color and of gelatinous consistency. The cortex overlying the areas of medullary involvement may be extremely thin or completely eroded so that the tumor extends through the periosteum into the surrounding soft tissues. Histologically the tumor is found to be very cellular and composed almost entirely of one type of cell. A fine fibrous reticulum supports masses of these cells in various stages of development, more immature forms being found in those with a high degree of malignancy. As was stated above, a vast majority of these tumors are composed of plasma (myeloma) cells, while those composed of erythroblasts and myeloblasts are extremely rare.

The relationship between the myeloblastic type of multiple myeloma and myelogenous leukemia is interesting and intriguing. The predominating cell and the histologic picture within a myeloblastoma are similar to those encountered in the bone marrow of leukemic patients, except that in myeloma the lesions are localized whereas in leukemia there is generalized involvement of the marrow. The interrelationship between solitary myeloblastoma, multiple myeloblastoma, aleukemic myelogenous leukemia, and ordinary myelogenous leukemia is not understood. Similarly it is found that multiple myeloma of the plasma cell type may occasionally be associated with the peripheral blood picture of plasma cell leukemia.

Symptomatology

Pain is one of the most outstanding symptoms of multiple myeloma. It may be localized directly over an area of bone involvement or referred to a neighboring joint; it may be an intense, constant pain or intermittent in character. It is most frequent in the thorax and in the lower lumbar region and its onset is commonly attributed to some slight trauma. The pain is usually aggravated by motion and exertion, and is relieved by rest. A tumor mass may sometimes be felt arising from bone in the painful area, but this is relatively infrequent. Occasionally there is extreme superficial tenderness in the affected area but this too is not constantly present. Many of the lesions are asymptomatic and roentgenograms show extensive bone involvement in regions where there has been no pain or tenderness whatsoever. Weakness, which accompanies the severe progressive anemia, may be the only symptom. Pathologic fractures, particularly of the ribs, are frequent and may be the first indication of illness. Abnormal bleeding, usually of the nose or gums, is sometimes encountered.

A progressive anemia, presumably of myelophthisic origin, is a constant feature of multiple myeloma, although if discovered in the early stages of the disease the anemia may as yet be relatively mild. Its severity progresses as the erythropoietic marrow is displaced and anemia may be the predominant manifestation of the disease. The leukocyte count is extremely variable, ranging from a distinct leukopenia to a leukocytosis of 100,000 or more. In most instances there are no significant alterations in the differential count, although a few immature cells of the myeloid and erythrocytic series may occasionally be encountered. Myeloma or atypical plasma cells gain access to the peripheral blood stream in some patients, and in rare instances are so numerous as to suggest a diagnosis of plasma cell leukemia. The presence of these cells in the peripheral blood is so infrequent that they are rarely of help in establishing a diagnosis although, when encountered, they are of significance.

Sternal aspiration is an extremely valuable diagnostic aid. Even though there is no roentgenographic evidence of involvement of the sternum the material which is aspirated will contain the myeloma cells in a high percentage of cases. A negative result does not exclude the possibility of multiple myeloma as the bone marrow involvement may be irregularly distributed rather than diffuse, and repeated aspirations may be necessary. The plasma cells encountered on bone marrow smears do not have the same appearance as in histologic sections. There is considerable variation in their size and frequently the cell is very irregular in outline with one or several "pseudopodia-like" projections of the cytoplasm. The cytoplasm is basophilic, but stains less deeply than in the plasma cell as seen in tissue sections. It is frequently vacuolated, occasionally contains azurophilic granules, and may be blotchy or irregular in its staining reaction. The nucleus does not usually show the typical "cart wheel" or "clock face" distribution of chromatin but presents a more irregular distribution, although a tendency for the chromatin to be clumped around the periphery of the nucleus is frequently observed. The nucleus may be centrally placed or eccentric, mitotic figures are not uncommon and occasionally there may be more than one nucleus. Plasma cells do not entirely replace the normal marrow cells, but usually about 50 per cent are of the plasma cell type although occasionally the percentage is much higher. It has been stated that a diagnosis of multiple myeloma can be made with more certainty from the findings of sternal aspiration than from the roentgenologic findings.

The roentgenologic appearance of the involved bone is variable, but the most frequent findings are multiple circumscribed osteolytic lesions, which

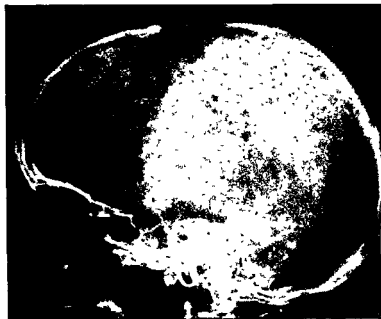


FIG 74 Roentgenogram of skull showing multiple small osteolytic lesions in a case of multiple myeloma.

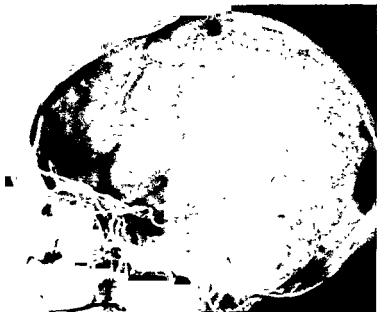


FIG 75 Roentgenogram of skull showing three sharply defined osteolytic lesions in a case of multiple myeloma.

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Albumin and casts are found in the urine, and evidences of renal insufficiency ultimately appear in most of the patients. There is a lowering of this renal function, an elevation of the nonprotein nitrogen of the blood, and the appearance of other symptoms and findings of chronic nephritis.

Diagnosis

The diagnosis of multiple myeloma is based primarily upon the presence of osteolytic bone lesions and a progressive anemia in a patient complaining of weakness and pain in the bones or joints. Hyperproteinemia, high blood calcium, and Bence Jones proteinuria are of help in establishing the diagnosis but may not be present in the early stages of the disease. The pathologic cells which comprise the tumor may be present in the material aspirated by sternal puncture. This procedure may give sufficient evidence to firmly establish the diagnosis. A biopsy may be taken from an accessible lesion if the diagnosis is still in doubt.

Multiple myeloma must be differentiated from metastatic malignant tumors of the bone, hyperparathyroidism, and other osteolytic types of bone lesions.

The disease is uniformly fatal with progressive weakness, cachexia, and anemia. The average duration of life after the onset of symptoms is about two years.

Treatment

Irradiation may relieve the pain of multiple myeloma but does not appreciably alter its course. The bone lesions are not altered in their roentgenologic appearance by this treatment. Transfusions are of value for the subjective improvement which they bring about although the effects are transient.

Stilbamidine given in conjunction with a diet low in animal protein is reported by Snapper to relieve the pain in most cases of multiple myeloma. This treatment checks the disease and relieves the pain but does not cure and does not influence the roentgenologic changes nor alter the sternal marrow findings except for the development of basophilic inclusion bodies within the cytoplasm of the myeloma cells. The drug is given intravenously, 50 mg. dissolved in 10 cc. of distilled water as the first dose, 100 mg. as the second dose after a two-day interval and thereafter 150 mg. every other day until a total of 4 to 5 Gm. has been given.

Solitary Myeloma

Solitary myelomas have been reported but these are probably only a different phase of the same disease, and the finding of a single lesion on roentgeno-

may occur in any bone but are most common in those areas containing hematopoietic marrow. These osteolytic lesions appear as sharply demarcated areas of rarefaction which have a



FIG. 76 Multiple myeloma. Multiple osteolytic lesions in the bones of the leg.

“punched-out” appearance (Figs. 74, 75, 76). There may be generalized osteoporosis of bone occurring either with or without circumscribed osteolytic lesions. In other cases there is generalized mottling of the bones. A roentgenogram of the skull should be taken in all patients in whom multiple myeloma is suspected as lesions are more likely to be visualized in this location than elsewhere.

Autoagglutination of erythrocytes is a striking feature in many cases of multiple myeloma. It may occur to such an extent that it interferes with the enumeration of erythrocytes and may cause rouleau formation on the blood smears. Rouleau formation may also interfere with typing and cross matching of the blood in preparation for blood transfusions. Warming or diluting the serum will sometimes prevent this reaction. The sedimentation rate is exceedingly rapid as the erythrocytes

clump together, imparting a granular appearance to the suspension, and settle very quickly.

Hyperproteinemia is found in a high percentage of patients with multiple myeloma. Values up to 20 Gm. per 100 cc. have been recorded. The increased protein content of the blood plasma is due to an increase in the globulin fraction, causing a reversal of the albumin globulin ratio. The hyperproteinemia is responsible for the increased sedimentation rate and autoagglutination. The serum calcium is frequently increased, but the serum phosphorus and phosphatase are normal or but slightly increased.

Bence Jones protein is found in the urine of about 75 per cent of the patients. It forms a white cloudy precipitate which appears when the urine is heated to between 50 and 60 C. and disappears at higher temperatures. Bence Jones protein has been found in other diseases which involve the bone marrow and is not pathognomonic of multiple myeloma.



FIG 77. Roentgenogram of the skull of a child with Gaucher's disease showing a large osteolytic defect.



FIG. 78 Roentgenogram of the skull of a child with Gaucher's disease showing several small osteolytic lesions

logic examination does not necessarily mean that it represents a solitary process. Many apparently solitary lesions ultimately become generalized, and sternal aspiration will frequently reveal that what appears to be a solitary myeloma is actually multiple. The solitary lesions have a better prognosis in so far as longevity is concerned and some are actually benign tumors. Extramedullary myelomas may occasionally be encountered.

LIPOID DYSTROPHIES OR LIPOID GRANULOMATOSIS

The reticulo-endothelial tissues are subject to damage by a group of diseases characterized by a disturbance of lipoid metabolism. These lipoid dystrophies have been subdivided into separate clinical entities which differ slightly in their clinical picture and in the chemistry of the abnormal lipoids. They are all manifestations of a similar metabolic disorder. The principal finding in so far as the hematologic picture is concerned is an anemia of myelophthitic origin due to displacement of normal bone marrow tissue by reticulo-endothelial cells containing abnormal metabolic substances.

Gaucher's Disease

Gaucher's disease is a rare familial condition characterized by an extreme degree of splenomegaly, chronic anemia, and the presence of large "foam cells" in the reticulo-endothelial system.

The spleen is large, firm, and pale red in color. On cut section it presents a granular surface with yellow or grayish red spots. Microscopic examination reveals that the pulp is almost entirely replaced by large pale cells with small eccentric nuclei. The cytoplasm is abundant and faintly staining and on chemical analysis is found to contain kersin. Similar cells are encountered throughout the entire reticulo-endothelial system, they have been called "foam cells" because of the appearance of their cytoplasm. These cells may be found in the material aspirated from the sternal marrow and also in that obtained by splenic puncture.

The disease is familial in incidence and chronic in its course. It begins in infancy or childhood although the symptoms may not become manifest until much later in life. The disease will pursue an especially chronic course when the manifestations do not become apparent until the second or third decade, but the course is more rapid when the onset occurs in infancy. The outstanding clinical manifestation is an extremely enlarged spleen. This increases in size very slowly and causes a heavy dragging sensation in the left

appear. Epistaxis, bleeding gums, and purpura are the most frequent hemorrhagic symptoms.

The course of the disease in infants and children may be quite rapid with progressive weakness, pallor, and cachexia. In older children and adults its progression is apt to be very slow with only mild fatigability accompanying the enlarged spleen. It is ultimately fatal.

Splenectomy may be performed to relieve the discomfort caused by the splenomegaly. This may give considerable subjective improvement but it does not significantly alter the course of the disease. Deep roentgen ray therapy has apparently caused some improvement, but the results are not striking. The treatment is otherwise purely symptomatic and supportive.

Niemann-Pick Disease

Niemann-Pick disease is a form of lipid dystrophy which appears earlier in life than Gaucher's disease. Its course is more rapid. It is congenital and familial in incidence and has been found predominantly in Jewish females. The spleen and liver become enlarged, and there may be lymphadenopathy. Gastrointestinal disturbances are a prominent part of the clinical picture and lead to a poor nutritional state. The skin becomes pale and edematous, and a brownish pigmentation appears. The blood cholesterol is elevated in some of the patients. Large cells similar to those described in Gaucher's disease are present throughout the reticulo-endothelial tissues and may be found in the material aspirated from the sternal marrow. In Niemann-Pick disease these cells give a positive reaction for lipid and on chemical analysis are found to contain a phosphatide rather than kersin.

A moderate degree of anemia is caused by the lipid-containing cells replacing the hematopoietic bone marrow. A leukocytosis is more commonly encountered than leukopenia. The platelets may or may not be affected.

The prognosis is hopeless, and no treatment has been found to be effective. Niemann-Pick disease is more rapid in course than Gaucher's disease, and death usually occurs within two months of the onset of symptoms. Splenectomy is of no benefit.

Hand-Schuller-Christian Disease

Hand-Schuller-Christian disease is a disorder of lipid metabolism appearing in childhood, frequently during the first two years of life. There are no racial or familial tendencies. The outstanding manifestations consist of exophthalmus, diabetes insipidus, and large yellowish softened areas in the skull and other membranous bones.

upper quadrant of the abdomen. Sharp pain may result from an infarction of the organ. The spleen may almost fill the left side of the abdominal cavity, and it is surprising that in such cases it does not cause greater difficulties. The liver becomes moderately enlarged, but there is no appreciable lymphadenopathy. There is a slight brownish discoloration of the skin in many of the patients. Yellowish wedge-shaped thickenings—pingueculae—appear in the conjunctiva on both sides of the cornea in older patients. Roentgenograms may show either a *generalized rarefaction and thinning of the cortex*

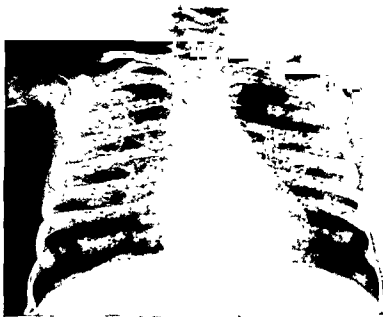


FIG 79 Roentgenogram of the chest of a child with Gaucher's disease showing a diffuse pulmonary infiltration.

of the bones or sharply demarcated osteolytic lesions (Figs. 77, 78). Pain is occasionally noted over the area of bone involvement. In some cases a diffuse infiltration of the lungs has been noted which is suggestive of military tuberculosis in its appearance (Fig. 79). The blood cholesterol may be increased but is usually within normal limits

The anemia is usually moderate in degree, and the erythrocytes appear normal or but slightly hypochromic. This anemia is due primarily to displacement of the normal cellular elements of the marrow by kersin-containing cells. Leukopenia is a characteristic feature of the disorder, the granulocytes being reduced so that a relative lymphocytosis results. There may be a reduction in the number of platelets so that hemorrhagic tendencies

a marked reduction in number of erythrocytes, leukocytes, and platelets. Death resulted from profuse hemorrhages, a complication of the thrombopenia.

The yellowish nodules and diffuse infiltrative lesions of the skin present a striking appearance. The cholesterol and total lipid levels of the blood plasma may be extremely high. There are no hematologic changes in the benign or localized forms.

Letterer-Siwe Disease

Letterer-Siwe disease is a nonlipoid reticulo-endotheliosis which affects infants. The bone lesions on roentgenologic examination are identical to those of Hand-Schuller-Christian disease, but histologic examination shows little or no lipid material present. Lymphadenopathy, splenomegaly, hepatomegaly, bone defects, and cutaneous lesions are present. The disease is usually fatal.

Eosinophilic Granuloma

Eosinophilic granuloma is a disease of children and young adults in which there are single or multiple bone lesions due to a reticulo-endotheliosis. The pathologic picture is similar to that of early Hand-Schuller-Christian disease. It is possible that this disease is merely one stage in the development of a lipid dystrophy.

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There is extensive hyperplasia of the reticulo-endothelial cells and widespread infiltration by large cells of this system which contain cholesterol. In many cases there is more extensive fibrosis in the hematopoietic portion of the bone marrow than is encountered with other lipoid dystrophies. Roentgenologic examination reveals areas of rarefaction in the bones which, on histologic examination, are found to be composed of degenerated cholesterol-containing cells. Exophthalmus is caused by lesions in the orbital bones and diabetes insipidus by lesions of this type in the region of the pituitary gland. There may be actual invasion of the pituitary gland itself as well as deformities of the sella turcica. The peribronchial and perivascular tissues of the lung may become densely infiltrated with the cholesterol-containing cells. A diffuse fibrosis results which simulates the appearance of miliary tuberculosis on roentgen ray examination. This may be the most important of the visceral lesions and may be the cause of death. Cutaneous lesions are frequently encountered and consist of papular, pustular, or hemorrhagic eruptions on a dry, yellowish skin. These have been shown to be due to an infiltration of the skin by the lipoid-containing cells. The blood cholesterol may be increased in the late stages of the disease.

Headaches, drowsiness, and irritability are common symptoms. There may be severe pain and tenderness over the bones of the extremities. The gums may be swollen and the teeth loosened. The liver and spleen are usually but not always enlarged, never to an extreme degree. The lesions in the bones dominate the clinical picture of this disorder. The course of Hand-Schüller-Christian disease is usually more chronic than that of Niemann-Pick disease but more rapid than Gaucher's disease. The anemia is mild in the early stages but becomes progressively more severe as the disease advances and may be accompanied by leukopenia and thrombopenia. The blood picture is suggestive of aplastic anemia when the leukocytes and platelets are reduced.

Roentgen ray therapy to the liver, spleen, and other involved areas, particularly the bone lesions, has proved to be of temporary benefit but does not alter the course of the disease.

Xanthomatosis

Orange or yellowish lipoid material may be deposited in localized areas or generally throughout all of the cells of the reticulo-endothelial tissues. This condition is known as xanthoma or xanthomatosis. In the generalized form of the disorder the involvement of the bone marrow may be extensive enough to produce a severe myelophthisic anemia. We have encountered one patient in whom there was a hematologic picture of aplastic anemia with

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HEMATOLOGY IN INFANCY AND CHILDHOOD

SINCE THE NORMAL BLOOD FINDINGS AND THE RESPONSE OF THE BLOOD TO various diseases during infancy and early childhood differ from those of the adult, special consideration must be given to interpretation of changes in the blood at this age. The hematopoietic organs of the infant are more labile and more active than those of the adult so that there is a more rapid and more intense response to an infection or other stimulus. Because of this hyperreactivity nucleated erythrocytes are commonly found in an infant's blood as a manifestation of regeneration whereas a similar stimulus in later life would cause only slight polychromatophilia. Infection or other stimulus to leukocytes may result in the appearance of myelocytes, metamyelocytes, and a hyperleukocytosis in place of the adult reaction of a moderate leukocytosis and an increase in band neutrophils. This hyperactivity gradually subsides so that the hematopoietic reactions at the age of puberty are essentially the same as in the adult. These peculiarities of the hematopoietic response during childhood must be borne in mind in order to evaluate properly the hematologic changes which occur.

Hemoglobin

The blood hemoglobin is higher at birth than at any subsequent time, but there is a rapid decrease during the first weeks of life. The amount of hemoglobin per unit of blood in infants is variable, and there are variations in the rapidity and extent of the subsequent decrease, the latter being influenced by environmental factors and differences in the methods of feeding infants. The high hemoglobin level at the time of birth is a result of the low oxygen tension which has prevailed in utero. With the beginning of respiration the availability of oxygen is greater and the need for excessive amounts of hemoglobin disappears. The blood hemoglobin level falls rapidly, and there is a decrease in the number of erythrocytes. This destruction of erythrocytes and liberation of their pigment accounts for the physiologic icterus of the newborn.

The blood hemoglobin at the time of birth averages between 22 and 23 Gm per 100 cc. of blood. This decreases rapidly so that by the tenth week there remain but 11 to 13 Gm. per 100 cc. of blood, which level is main-

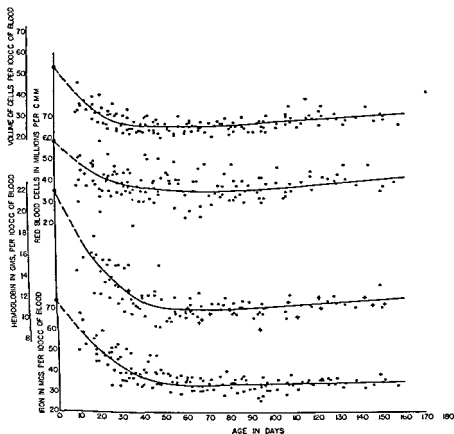


FIG 80 Showing the decrease in the hematocrit, erythrocyte count, hemoglobin, and blood iron during early infancy. The values were obtained from repeated analyses of blood of seventeen infants between 10 and 160 days of age. The average values at birth are estimated, the curves are drawn from the average values obtained at the different ages. Cell volumes and hemoglobin are shown in percentages, red cells in millions per cubic centimeter, iron in milligrams per 100 cc. of blood.

tained throughout the next two years. There is a gradual but slow increase after 2 years of age, and the adult level is reached at about the sixteenth year. The hemoglobin level during this period is subject to minor fluctuations and individual variations but in general follows the above course. Curves showing

the decrease in hemoglobin, red blood cells, hematocrit, and blood iron during early infancy were prepared by Stearns and are shown in Figure 80. She has shown that the blood hemoglobin in breast-fed babies does not drop to as low a level as in artificially fed infants and that the addition of iron or iron-containing foods aids in maintaining a higher hemoglobin level. The amount of hemoglobin in the newborn is high in relation to the number of erythrocytes since the average erythrocyte is larger than normal. There is no demonstrable difference in the blood values between the two sexes at this age.

Alt has shown that removal of blood from the umbilical cord at the time of birth or the early tying of the cord deprives the infant of needed hemoglobin and erythrocytes and causes a significantly lower hemoglobin level and erythrocyte count during the first weeks of life. This procedure is equivalent to subjecting the infant to a severe hemorrhage and may lead to an iron deficiency during the first year of life. The mean corpuscular hemoglobin is lower at the age of 8 to 10 months in those infants whose umbilical cords were clamped immediately after birth than in those in whom tying of the cord was delayed.

Erythrocytes

The erythrocyte count is high at birth and has been found to average 5,850,000 cells, some observers reporting considerably higher values. There is a great fluctuation during the first days of life, but the count declines to an average level of 3,920,000 at 6 weeks and reaches the lowest average of 3,400,000 at 11 weeks. There is a subsequent rapid increase in the number of erythrocytes to about 4,000,000 and then a gradual increase to adult levels, the rise in number of erythrocytes paralleling that of the hemoglobin values.

There is some variation in the size of the erythrocytes as observed on the smear, but the average diameter is greater than in the normal adult, being from 8.5 to 9 microns. The variations in the size of the cell disappear within a few days and the macrocytosis soon disappears. Hematocrit values have been found to average 54 per cent at the time of birth, to decrease to 24 per cent by the eighth week and then gradually to increase to between 30 and 40 per cent by the thirty-seventh week of life.

Nucleated erythrocytes are frequently encountered in the blood of a newborn infant, from 1 to 2 nucleated erythrocytes per 100 leukocytes usually being present although larger numbers are not infrequent. These

are usually normoblasts, but more immature forms may occasionally be encountered. They disappear from the blood stream within a few days.

Reticulocytes are present in fairly large numbers, frequently reaching from 6 to 7 per cent at the time of birth, but they gradually decrease to 1 per cent or below by the end of six weeks.

The platelets are not significantly altered as compared to adult values but their number tends to be somewhat high immediately after birth and to remain elevated during the first few years of life.

Leukocytes

The total leukocyte count is high at birth, most observers finding it above 15,000 per cubic millimeter of blood. Occasionally it is as high as 25,000. Repeated counts during the first hours and days of life show moderate fluctuations, but in general there is a decline which reaches its minimum on about the fifth day as is shown in the accompanying table compiled by Wollstein. Following this there is an increase to around 9000 to 12,000 where the count remains during the first year of life although there may be rather marked daily variations which appear to be independent of feeding, exertion, or other exciting factors. The leukocyte count does not fall to the normal adult level until about the tenth year.

WHITE CELLS FROM BIRTH TO FIFTEEN DAYS

Age	Total Leukocytes	Total Lymph.	Per Cent Lymph	Total Mon	Per Cent Mon	Total Eosin.	Per Cent Eosin.
1st day	14,400	3456	24	144	1	288	2
2nd day	13,000	3120	24	130	1	260	2
3rd day	11,700	3159	27	234	2	468	4
4th day	7360	2796	38	73	1	294	4
5th day	7100	2911	41	0	0	426	6
7th day	7320	3001	41	146	2	365	5
8th day	8850	5221	59	88	1	176	2
9th day	9700	4248	44	291	3	291	3
10th day	7520	3524	47	75	1	225	3
11th day	9700	5141	53	582	6	0	0
15th day	9550	4584	48	0	0	475	5
Adult	6000 to 7000	1500 to 2000	20 to 30	200 to 350	3 to 6	100 to 200	1 to 3

Immediately after birth there is an increased percentage of neutrophils on the differential count, but during the first week or two this gradually recedes to an abnormally low level so that neutrophils may comprise only

20 to 40 per cent of the white blood cells. There is an increase in the number and percentage of lymphocytes so that they usually exceed the neutrophils throughout the first six years of life. Eosinophils and basophils may be unusually high at birth but decrease with the neutrophils. The number of monocytes tends to parallel the lymphocytes, being rather low at birth but increasing to relatively high percentages at about 2 weeks of age.

Immature white cells are not infrequently encountered on the smear during early infancy so that an occasional myelocyte or metamyelocyte during the first few days of life is rather common. They are seldom found after the tenth day. The percentage of band or nonfilamented neutrophils is likewise high at birth, frequently ranging from 30 to 40 per cent, but decreases to the normal adult level of 4 to 5 per cent by the end of two weeks. Large and relatively immature lymphocytes are also more common in infants' blood than in adults'.

PATHOLOGIC BLOOD PICTURES IN INFANCY AND CHILDHOOD

These physiologic variations in the hemoglobin, erythrocytes, and leukocytes must be taken into consideration when interpreting the blood picture of infants and children. Likewise the hyperreaction of the hematopoietic system to various stimuli must be borne in mind as the blood picture in a child may be considerably different from that encountered in an adult with a similar disease. A roentgenologic examination of the skeletal system, especially the skull, is often a particularly valuable aid in the diagnosis of hematologic disease in children. Under the demand for excessive blood formation in children, as in the hemolytic anemias, there is an expansion of the bone marrow with absorption of the trabeculae of the marrow and a thinning of the cortex. Perpendicular spicules of bone radiating outward from the periosteum have also been encountered. This overgrowth of the marrow is particularly striking in Cooley's (Mediterranean) anemia and the details of the bone changes are described in more detail in Chapter XIII. Similar changes are encountered in other forms of hemolytic anemia which have their onset in infancy. The lipid dystrophies which appear in childhood produce typical osseous defects and osteosclerosis with its thickening of the cortex and reduction of the medullary space also makes its appearance at this age. Both of these conditions may produce a severe anemia. Bone changes may be evident in children with myelogenous leukemia whereas such roentgenologic features are not common in the adult with this disease. These have been discussed in Chapters X and XIII respectively.

Anemias

The anemias of infancy and childhood have been discussed in the appropriate sections on anemia, but certain features may be re-emphasized at this point. Anemia in the newborn infant is seldom encountered except in rare cases of congenital hypoplastic anemia and erythroblastosis. In the latter condition there are extremely large numbers of nucleated erythrocytes in the blood stream together with a marked degree of polychromatophilia and diffuse basophilia. This response is similar to but much more intense than that of an adult with severe and rapid hemolysis of the erythrocytes.

Other forms of the hemolytic anemias which are discussed in Chapter XIII may appear in early childhood. These include familial hemolytic icterus, sickle cell anemia, Lederer's anemia, and Cooley's anemia. The essential features are the same as in adults except for a more intense reaction in infancy. Nucleated erythrocytes are frequently found in an infant's blood when only polychromasia or a reticulocytosis would be noted in the adult, and the polychromasia which develops in the infant is even more marked than in the adult. Such polychromasia is apt to be a striking feature in any type of infantile anemia except the aplastic type. Nucleated erythrocytes may appear in appreciable numbers in response to any rather mild grade of anemia in which there is compensatory regeneration of erythrocytes. The hemolytic anemias are characterized by a leukocytosis which is particularly striking when the disease occurs in infants. Immature cells appear in the peripheral blood so that myelocytes, metamyelocytes, and band neutrophils may be numerous. This leukocytic response to an anemia, particularly the hemolytic type, has led to the erroneous use of "pseudoleukemia" in describing the disease.

Megaloblastic anemia Although true Addisonian pernicious anemia does not occur in childhood there is a somewhat similar type of anemia in children, from one to eighteen months of age, the pathogenesis of which has not as yet been determined. This is characterized usually by a macrocytosis, but with a wide variation in the diameter of the erythrocytes, and by the presence of some nucleated erythrocytes. It tends to be normochromic rather than hyperchromic. There is a reduction in the leukocyte count with hypersegmented neutrophils and giant metamyelocytes in the peripheral blood. The platelets are reduced and this is often associated with hemorrhagic tendencies and purpura. The bone marrow is similar or identical to that found in pernicious anemia with a megaloblastic reaction and many abnormal young granulocytes. An achlorhydria is commonly present but does not persist after the anemia is cured. Splenomegaly is commonly but not consistently present. A history

of a preceding episode of vomiting, anorexia, fever, and upper respiratory infection is commonly obtained. The nutritional history reveals inadequacies in many but not all of the cases and it is probable that this anemia is the result of a nutritional deficiency or infection. It responds promptly to the administration of folic acid, from 5 to 20 mg. per day, with a reticulocyte increase, a reversion of the bone marrow to normal, and a steady rise in the hemoglobin and erythrocyte levels. No relapses have been observed.

Aplastic anemia is not common in childhood and only rare cases of congenital hypoplastic anemia are encountered. Most of the cases in which the blood picture is suggestive of aplastic anemia eventually turn out to be leukemia in an aleukemic phase, a disease which is not infrequent in childhood and must be considered in the differential diagnosis of all unexplained progressive anemias. Examination of the sternal marrow should be done in all such cases as the leukemic infiltration is diagnostic.

Iron deficiency anemias (Chapter IX) are not present at birth but tend to develop during the first year of life, commonly after the fourth month. Even when the mother has a severe grade of hypochromic anemia, the infant will have a normal amount of hemoglobin at birth although there is a greater tendency for this infant to develop a hypochromic anemia during the first year of life than for a child born of a normal mother.

The *anemia of prematurity* is primarily an iron deficiency anemia and is described more fully under that heading. It is not present at birth but develops during the first year of life when the demand for iron by the rapidly growing child is particularly great. Since the iron stores of the body have not been adequately filled before birth, the available iron is soon utilized and typical hypochromic anemia develops. It is aggravated by an inadequate iron intake and can be corrected by the addition of a simple iron salt to the child's feeding. The blood volume of such infants is less than normal at the time of birth so that the source of material for new blood formation, the products of blood destruction, is not adequate to meet the needs of the rapidly growing infant. It has been suggested, but not proven, that the hematopoietic system of these infants is not fully developed and that this may contribute to the anemia.

Even though the iron stores of an infant are adequately filled at the time of birth, they may become depleted during the period of rapid growth if not adequately replenished by the diet. *Nutritional anemia of infants* may occur if the child is kept on a diet composed exclusively of cow's milk for too long a period or if supplemental foods are not added to the diet at the proper time. A careful dietary history should always be obtained from the parents in any

case of unexplained anemia in childhood. An adequate diet may quickly correct a severe anemia which has developed on this basis.

Celiac disease may interfere with the absorption of iron from the intestinal tract and so lead to a typical hypochromic anemia. A macrocytic hyperchromic anemia is found in other instances of celiac disease, especially when the disease is of long standing. It is thought that this type of anemia is due to faulty absorption of the maturation factor. It can be corrected by the administration of autolyzed yeast, from 2 to 4 Gm. per day.

Goat's milk anemia is a macrocytic anemia encountered primarily in Germany and Italy and appears to be a form of nutritional anemia. It is rare in this country. It occurs in infants fed exclusively on goat's milk and may be cured by liver extract, yeast, or an adequate diet.

Congenital hypoplastic anemia resembles aplastic anemia in the adult but is present at birth. It is a rare disease and responds only to repeated blood transfusions.

Von Jaksch's anemia is an ill-defined disease characterized by severe anemia in childhood associated with a marked elevation in the leukocyte count and the presence of immature granulocytes in the blood stream. The latter feature has led to the use of the term *infantile pseudoleukemia*. This disorder probably represents a severe anemia in early childhood which results from infection or from hemolysis of the erythrocytes and is accompanied by a hyperreactivity of the bone marrow with a resultant high leukocyte count. As pointed out in Chapter XIII, it would be best to drop the term *von Jaksch's anemia* since it does not seem to represent a specific disease entity.

Infection leads to the development of anemia in children as in adults. Acute infections seldom produce a severe degree of anemia although a rather rapid fall may occur in the hemoglobin and erythrocyte levels at the onset of the infection.

Chronic infections lead to varying degrees of anemia depending upon their severity, duration, and the causative organism. The anemia is due to interference with the nutritional state of the child, to hemolysis of erythrocytes, and to a toxic depression of the bone marrow. The disability which goes with a persistent *otitis media* may be due as much to the accompanying anemia as to the original infection. A search for a hidden infection must be undertaken in all obscure anemias.

Hemorrhagic diseases are not infrequent during infancy and childhood. Hemophilia is the most common of these and frequently makes its appearance early in life. Hemorrhagic disease of the newborn, due to vitamin K deficiency, is not uncommon. Thrombopenic purpura of the idiopathic type

of a preceding episode of vomiting, anorexia, fever, and upper respiratory infection is commonly obtained. The nutritional history reveals inadequacies in many but not all of the cases and it is probable that this anemia is the result of a nutritional deficiency or infection. It responds promptly to the administration of folic acid, from 5 to 20 mg. per day, with a reticulocyte increase, a reversion of the bone marrow to normal, and a steady rise in the hemoglobin and erythrocyte levels. No relapses have been observed.

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In bronchopneumonia the leukocyte count will frequently reach 30,000 to 40,000 with neutrophils predominating and, especially in younger children, a marked degree of immaturity of the cells.

Lobar pneumonia may be associated with a leukocyte count of 15,000 to 50,000 with a preponderance of neutrophils and many immature cells. A typical leukemoid reaction may result from this infection.

Empyema brings forth a very high leukocyte count, frequently from 20,000 to 40,000, with many immature cells.

Measles causes little or no increase in the total leukocyte count although a differential count shows a relative increase in neutrophils. A leukocytosis may be detected during the incubation period, but at the time of the catarrhal stage and skin eruption this has disappeared. A lymphocytosis rather than a neutrophilia may occur.

German measles does not cause a significant elevation in the leukocyte count, but plasma cells may become a prominent constituent of the peripheral blood.

Mumps may be associated with slight or no increase in the total leukocyte count. An actual leukopenia is sometimes encountered.

Meningitis, either meningococcic or due to other pyogenic organisms, results in a particularly high leukocytic response, from 30,000 to 50,000 cells being a common finding. There are various degrees of immaturity of the cells.

Mastoiditis results in a neutrophilic leukocytosis, frequently from 20,000 to 40,000 cells.

Otitis media causes a leukocyte response which is ordinarily not as high as in mastoiditis.

Influenza does not cause a leukocytosis. An actual leukopenia may develop with the count ranging from 3000 to 5000.

Osteomyelitis produces a variable response, but ordinarily the leukocyte count runs from 20,000 to 40,000 in the acute forms. Although this is usually a neutrophilic response, we have observed a lymphocytosis with this disease.

Pertussis, as already mentioned, produces an increase in the lymphocytes, which reach their greatest number during the paroxysmal stage. At that time a total leukocyte count of 20,000 or over is not uncommon.

Pyogenic abscess results in a neutrophilic leukocytosis of varying degree, the height of the count depending upon the type of organism and the location of the abscess. A count of 15,000 to 20,000 is not uncommon, and varying degrees of immaturity of the cells will be encountered. A similar neutrophilic leukocytosis will be observed with an acute appendicitis.

Poliomyelitis leads to a variable blood picture, with usually a moderate

is rare but may be present at the time of birth or appear during the early months of life. Profuse hemorrhage from any of these conditions will produce a severe degree of anemia.

Leukemia

Congenital leukemia, which is detectable at the time of birth, is exceedingly rare, but it is not uncommon for leukemia to make its appearance during the first year of life (Chapter XVII). We encountered 3 such cases among 61 children with leukemia. Lymphocytic leukemia is by far the most common type in childhood and is practically always of the acute type. Aleukemic forms comprise about a third of the cases. Myeloid leukemia is seldom seen in infancy but occasionally appears in later childhood although it is far less common than the lymphocytic type even then. Various types of infectious diseases in infancy may cause extremely high leukocyte counts with the appearance of myelocytes and metamyelocytes in the peripheral blood. The possibility of such a leukemoid reaction must therefore always be borne in mind when these blood findings are encountered in children.

Infections

An infection in infancy or childhood may occasionally cause a lymphocytic response rather than the usual increase in neutrophils. Such a lymphocytosis is to be expected in pertussis, when the total white count may reach 40,000 with a preponderance of lymphocytes, but it is occasionally encountered in other types of infectious diseases. We have found a leukocyte count in excess of 50,000 with 90 per cent lymphocytes in a case of osteomyelitis which, upon control of the infection, returned to normal.

The usual neutrophilic leukocytosis is encountered in most patients with infections, but the total count is usually higher. The percentage of immature cells is greater than would be expected in an adult with a similar infection. These so-called leukemoid reactions present troublesome diagnostic problems in many cases.

Leukocytic Response to Common Childhood Diseases

Chickenpox is usually associated with a relatively low leukocyte count, usually below 10,000, and a lymphocytosis.

Diphtheria causes a leukocyte count of 15,000 to 20,000 with an increase in neutrophils.

Acute bronchitis does not produce a marked rise in the number of leukocytes unless it is complicated by bronchopneumonia.

Chapter XXIII

TRANSFUSION OF WHOLE BLOOD AND BLOOD DERIVATIVES

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INTRODUCTION

THE TRANSFUSION OF BLOOD MAY BE REGARDED AS THE MOST SUCCESSFUL type of tissue transplantation so far developed. The history of its evolution is long and interesting but is not within the scope of the present chapter. Chemical and physiological knowledge has progressed to the stage where it is no longer practical to regard blood merely as individual cells suspended in tissue juice. Rather should it be considered as a complex mixture of proteins and crystalloids containing cells which, if not living, are at least physiologically active.

It is necessary to take cognizance of the composition of the plasma in order to consider intelligently the indications for, and the technic of, blood transfusion. Plasma is a solution of crystalloids and proteins, the latter in a concentration of 6 to 7 per cent. Three-fifths of the plasma protein is albumin, which exerts about 80 per cent of the total colloid osmotic pressure and is readily assimilable by the body. Most of the remainder of the protein fraction is composed of globulins of higher molecular weights, which are consequently less osmotically active. Intimately associated with the globulins are the isohemagglutinins, the antitoxins, and other immune bodies. Most of the factors concerned in the mechanism of coagulation of the blood reside in the plasma: prothrombin, fibrinogen, antithrombin, and calcium. Only thromboplastin is derived from cellular elements.

The erythrocytes may be regarded as sacs of hemoglobin enclosed by semipermeable membranes through which diffuse oxygen, carbon dioxide,

leukocytosis. However, counts of 20,000 or over may be noted. The white count returns to normal with subsidence of the febrile course.

Rheumatic fever causes a moderate or high degree of leukocytosis with neutrophils predominating.

Scarlet fever causes a leukocyte count of 15,000 to 30,000 with immature cells sometimes present and occasionally with 5 to 10 per cent eosinophils.

Subacute bacterial endocarditis does not cause a particularly high leukocyte count. This may range from 8000 to 15,000.

Fractures of larger bones may cause a surprisingly high degree of leukocytosis.

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Hemolysis results from the combination of red cells and hemolysins in the presence of complement so that the reaction cannot be demonstrated in bloods in which the complement has been inactivated by aging or by subjection to 56°C for thirty minutes.

Nomenclature of Groups

The four blood groups are named from the agglutinogens in the erythrocytes. They are designated by the capital letters *A*, *B*, *AB*, and *O*. The letter *O* was originally employed to represent zero, implying an absence of agglutinin, but it is now known that the agglutinin *O* is a specific substance, for which there is no naturally occurring agglutinin. The plasma of group *A* contains β or anti-*B* agglutinin; plasma of *B* contains α or anti-*A*; *AB* contains no agglutinins in the plasma; group *O* blood has both anti-*A* and anti-*B* agglutinins. These designations are according to the Landsteiner or International nomenclature and are now almost universally accepted because of the descriptiveness of the terms. They supersede the older numerical designations, which unfortunately persist in use in some institutions. The equivalent classifications are as follows:

International	Moss	Jansky
A	II	II
B	III	III
AB	I	IV <i>universal recipient</i>
O	IV	I <i>universal donor</i>

Inheritance

It has been firmly established that the blood groups of the ABO system are transmitted through inheritance according to the mendelian law. The mechanism by which this occurs has been extensively studied. The theory of Bernheim has now been widely accepted. This postulates the existence of three allelic genes, *A*, *B*, and *O*. Since each germ cell contains only one of the genes, there exists the possibility of combination between three kinds of sperms and three kinds of ova. *A* and *B* are dominant over *O*. Two laws of heredity have been derived from these considerations (1) A child cannot acquire an agglutinin which has not been present in the blood of one or both of the parents (2) Since *O* is recessive, it is impossible for a child to belong to this group if one of the parents is *AB*, and conversely, the combination of group *O* parent and group *AB* child is impossible. Only the hereditary combinations listed in the accompanying table are possible

chlorides, and dextrose. The human erythrocytes contain about eighteen times as much potassium as is found in the surrounding plasma. Although the concentration of sodium in the plasma is high, scarcely any can be found in the cells. The body under normal conditions forms about 1 per cent of the circulating erythrocytes daily and destroys a corresponding number. Any volume of blood, therefore, at the time of collection is composed of equal quantities of cells from 1 to approximately 100 days of age.

BLOOD GROUPS AND SUBGROUPS

The ABO System

Definition

All human beings may be classified as belonging to one of four chief blood groups. They are distinguished by the presence or absence of certain specific polysaccharides in the erythrocytes and in all body cells. These substances are designated by the capital letters A, B, and O. When present in the erythrocytes they are known as *isohemagglutinogens*; in other tissues or fluids they are referred to merely as *group-specific substances*. There are two chemical forms, the lipoidal and the water-soluble. All persons have the lipoidal form in their erythrocytes and other tissues. Most, in addition, have inherited the ability to produce the water-soluble form in quantity, and this is secreted in the saliva, urine, tears, semen, gastric juice, and milk. These individuals have been designated *secretors*; those lacking sufficient water-soluble fraction are called *nonsecretors*. It has been shown that the ability to secrete group-specific substances is inherited as a mendelian dominant character. It is of some practical interest that substances present in the stomach of the hog and in the capsule of Type I pneumococcus give reactions immunologically similar to that of the A group-specific substance. The saliva and gastric mucosa of the horse contain substances immunologically identical with both A and B substances.

Plasma may contain *agglutinins* which react specifically with the agglutinogens A and B of the red cells to produce clumping or agglutination. The agglutinin specific for the A substance is called *alpha* (α) or *anti-A*, and that specific for the B substance is termed *beta* (β) or *anti-B*. It follows that it is biologically impossible for the blood of one individual to possess an agglutinin and the specific agglutinin with which it reacts. In about 30 per cent of the plasmas containing an isohemagglutinin, *isohemolysins* are demonstrable, specific in all respects for the same group substance. The titer of the hemolysin is usually lower than that of the corresponding isoagglutinin.

Hemolysis results from the combination of red cells and hemolysins in the presence of complement so that the reaction cannot be demonstrated in bloods in which the complement has been inactivated by aging or by subjection to 56°C for thirty minutes.

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O	IV	I <i>universal donor</i>

Inheritance

It has been firmly established that the blood groups of the ABO system are transmitted through inheritance according to the mendelian law. The mechanism by which this occurs has been extensively studied. The theory of Bernheim has now been widely accepted. This postulates the existence of three allelic genes, *A*, *B*, and *O*. Since each germ cell contains only one of the genes, there exists the possibility of combination between three kinds of sperms and three kinds of ova. *A* and *B* are dominant over *O*. Two laws of heredity have been derived from these considerations: (1) A child cannot acquire an agglutinogen which has not been present in the blood of one or both of the parents. (2) Since *O* is recessive, it is impossible for a child to belong to this group if one of the parents is *AB*, and conversely, the combination of group *O* parent and group *AB* child is impossible. Only the hereditary combinations listed in the accompanying table are possible.

Groups of Parents	Groups of Children
O × O	O
O × A	O or A
O × B	O or B
A × A	O or A
A × B	O, A, B, or AB
B × B	O or B
O × AB	A or B
A × AB	A, B, or AB
B × AB	A, B, or AB
AB × AB	A, B, or AB

It is therefore impossible for an individual permanently to change blood groups. Instances apparently to the contrary are usually due to faulty determination of the agglutinogens in the cells.

Development

The agglutinogens are first detectable during the second month of fetal life. Thereafter up to the age of approximately 20 years the sensitivity of the agglutinogens increases. At birth the cells have attained about 20 per cent of the maximum potency. On the other hand, it is believed that no newborn infant possesses agglutinins of its own although some agglutinins are detectable in about 50 per cent of the cases. These are thought to belong to the mother and to have filtered through the placenta. They usually disappear during the first ten days of postnatal life. The agglutinins gradually increase in titer and attain their maximum between the tenth and twentieth year. Thereafter the potency gradually diminishes during the remainder of life. Most bloods containing both agglutinins have one in higher titer than the other, the anti-A usually being the stronger. The low sensitivity of the agglutinogens in young children readily accounts for the difficulties frequently encountered in the determination of the blood groups and the occasional errors in crossmatching associated with transfusions in pediatric practice.

Incidence

The incidence of the four blood groups is of practical importance when transfusion is under consideration. The proportionate occurrence of each group varies greatly in different races. This datum has been employed in anthropologic studies. The approximate incidence in the white population of the United States is O, 45 per cent; A, 40 per cent; B, 10 per cent; and AB, 5 per cent. In the American Negroes the distribution is approximately O, 45 per cent; A, 30 per cent; B, 20 per cent, and AB, 5 per cent. Nearly

half the population, therefore, are universal donors but only one-twentieth are universal recipients.

The Subgroups A_1 , A_2 , A_1B , and A_2B

If the cells from a number of group A bloods are combined with a potent anti-A serum, it will be noted that a few bloods are but weakly agglutinated in comparison with the majority. This difference can also be observed when group AB cells are tested with anti-A serum. The cells with weak sensitivity

POTENCY OF ISO-ANTIBODIES DURING LIFE

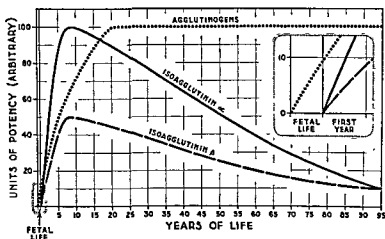


FIG 81 Semidiagrammatic representation of variations in potency of isoantibodies during life compiled from data from several sources

belong to subgroup A_2 or A_2B whereas the majority are classified as A_1 or A_1B . About 20 to 25 per cent of group A bloods belong to subgroup A_2 . The subgroups are usually not differentiated in routine grouping and cross-matching because the anti-A agglutinin in group B blood is, in reality, a mixture of anti- A_1 , and anti-A. The cells of both subgroups are therefore agglutinated and termed *group A*. The practical importance of the subgroups in groupings is that the lesser sensitivity of the A_2 agglutino-gen sometimes results in the erroneous conclusion that an A_2 blood is an O or that an A_2B is a B. This error can usually be avoided by employing anti-A grouping serum, which is known to be potent against A_2 agglutinogens.

In 1 to 2 per cent of group A_2 and in one-quarter of the bloods of group

Groups of Parents	Groups of Children
O × O	O
O × A	O or A
O × B	O or B
A × A	O or A
A × B	O, A, B, or AB
B × B	O or B
O × AB	A or B
A × AB	A, B, or AB
B × AB	A, B, or AB
AB × AB	A, B, or AB

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The Rh-Hr System

The blood of human beings may be further differentiated by another system of agglutinogens in the erythrocytes, the components of which are inherited independently of the ABO, the MN, or the P systems. Since there are no corresponding natural agglutinins, the agglutinogens can be demonstrated only by the action of immune human sera or, to a limited extent, by immune animal sera. When first discovered by Landsteiner and Wiener¹ the system was conceived as a simple one determined by the presence or absence of a single factor. This was named the *Rh* agglutinogen because it was first demonstrated with serum from rabbits immunized by injections of erythrocytes from the Rhesus monkey. The cells of about 15 per cent of white persons were found to be unagglutinable by this serum and were termed *Rh* negative whereas the 85 per cent, whose cells were acted upon, were designated as *Rh* positive. Agglutinins were soon found in immunized human beings which gave reactions at variance with this conception and the group of Rh-positive individuals was divided into a number of types. Three definitive agglutinating sera were designated by Wiener² as *anti-Rh₀*, *anti-rh'*, and *anti-rh''*. The cell types were named from the agglutinogens which they contain, according to the following scheme:

CLASSIFICATION OF Rh TYPES

Cell Types	Antisera			Incidence in Caucasians (Percentage)
	<i>Anti-Rh₀</i>	<i>Anti-rh'</i>	<i>Anti-rh''</i>	
rh (Rh neg)	—	—	—	13.0
Rh ₀	+	—	—	2.0
Rh ₁ (Rh ₀ ')	+	+	—	54.0
Rh ₂ (Rh ₀ '')	+	—	+	15.0
Rh ₁ Rh ₂ (Rh ₀ '')	+	+	+	14.5
rh'	—	+	—	1.0
rh''	—	—	+	0.5
rh'rh''	—	+	+	0.01

Caucasians, anti-rh' serum
The combination serum
about 86.0%.

The nomenclature of Wiener is not difficult to master. The symbol for Rh negative is rh (verbally, "little r-h" or simply "r-h"). The types agglutinated by anti-Rh₀ serum are marked by subscripts 0, 1, or 2, depending on whether they react with anti-rh' or anti-rh''-serum also. The subscripts 1 and 2 are shortened forms for the combinations 0' and 0''. The types which are not

A₂B there is found an anti-A₁ agglutinin which makes these bloods incompatible with the other subgroup. Very rarely in A₁, B, or A₁B serums an *anti-O* agglutinin occurs which reacts with all O cells and about 95 per cent of A₂ cells. This is sometimes called *anti-A₂* agglutinin. These anomalous agglutinins are discovered if routine crossmatching of bloods is employed before transfusion. The usual procedure when two group A bloods are found to be incompatible is to match that of the prospective recipient with a group O blood and to accept the latter if compatible. If the serum agglutinates group O cells, a blood of the same subgroup must be found.

The MN System

Definition and Significance

Besides the ABO system of agglutinogens contained in the human erythrocytes there are many others which can be demonstrated by special technics. Although valuable in the further identification of individual bloods in medico-legal problems, they are of little or no significance in blood transfusion because there are no naturally occurring specific agglutinins found in human plasma. One such system which has been extensively studied is that of the M and N agglutinogens. By testing with immune animal serums, all human bloods can be assigned to one of three groups depending on whether the cells contain M, N, or MN agglutinogens. The occurrence of these is independent of the ABO system. Only seven human serums have been reported as containing anti-M agglutinins; none have so far been found with anti-N factors.

Inheritance

Two laws of heredity have been worked out for this system (1) The factor M or N cannot appear in the child unless present in one or both of the parents (2) An M parent cannot have an N child and an N parent cannot have an M offspring. Only the following combinations are possible:

$$\begin{aligned} MN \times MN &= MN, M, \text{ or } N \\ MN \times N &= MN \text{ or } N \\ MN \times M &= MN \text{ or } M \\ M \times N &= MN \\ M \times M &= M \\ N \times N &= N \end{aligned}$$

By determining the M and N agglutinogens in addition to the A, B, and O, it is thereby possible to identify many more individual bloods than would be possible if only the latter agglutinogens were determined.

RH-HR GENOTYPES

Phenotypes Types	Antisera			Genotypes	Antisera			Phenotypes Subtypes	Incidence in Caucasians (Percentage)
	Anti- Rh ₀	Anti- rh'	Anti- rh''		Anti- Hr ₀	Anti- Hr'	Anti- Hr''		
rh	-	-	-	rr	+	+	+		13.0
rh'	-	+	-	r'r	+	-	+	rh'rh'	0.01
				r'r	+	+	+	rh'rh	1.0
rh''	-	-	+	r''r''	+	+	-	rh''rh''	0.005
				r'r	+	+	+	rh'rh	0.5
Rh'Rh''	-	+	+	r'r	+	+	+		0.01
Rh ₀	+	-	-	R ⁰ R ⁰	-	+	+		2.0
				R ⁰ r	+	+	+		
				R ¹ R ¹	-	-	+	Rh ₁ Rh ₁	20.0
Rh ₁	+	+	-	R ¹ r	+	-	+		54.0
				R ¹ r	+	+	+		
				R ¹ R ⁰	-	+	+	Rh ₁ rh	34.0
Rh ₂	+	-	+	r'R ⁰	+	+	+		15.0
				R ² R ²	-	+	-	Rh ₂ Rh ₂	
				R ² r''	+	+	-		
				R ² r	+	+	+		
				R ² R ⁰	-	+	+	Rh ₂ rh	12.0
Rh ₁ Rh ₂	+	+	+	r''R ⁰	+	+	+		14.5
				R ¹ R ²	-	+	+		
				r'R ²	+	+	+		
				R ¹ r''	+	+	+		

This table is slightly expanded from Wiener.³ It does not contain all the genotypes which are possible with the theory of eight allelic genes.

Fisher and Race⁴ conceive a somewhat different mechanism for the inheritance of the Rh antigens. They postulate three pairs of genes in closely related loci on a pair of chromosomes. One pair of loci is occupied by one of three gene combinations CC, Cc, or cc, another pair by the gene combination DD, Dd, or dd, and a third by the combination EE, Ee, or ee. The capital letters are the equivalent of three Rh genes whereas the lower case letters symbolize three Hr genes.

Equivalent Gene Nomenclature

Wiener	R'	R ⁰	R''	(H')	(H ⁰)	(H'')
Fisher,	C	D	E	c	d	e

The antibodies were given Greek letters by Fisher but this has proved quite confusing to many and the suggestion of Cappell has been widely accepted.

Equivalent Antibody Nomenclature

Wiener	Anti-Rh'	Anti-Rh ₀	Anti-Rh''	Anti-Hr'	Anti-Hr ₀	Anti-Hr''
Cappell	Anti-C	Anti-D	Anti-E	Anti-c	Anti-d	Anti-e

acted upon by anti-Rh₀ are marked with the superscripts ' or " to denote agglutination by anti-rh' or anti-rh" serum.

With the expansion of knowledge of the Rh types the definition of the terms Rh positive and Rh negative underwent a change. Actually one is now required to specify the serum to which the cells fail to react. For example, anti-Rh₀ serum classifies the types Rh' and Rh' Rh" as Rh negative whereas the use of anti-Rh₀' serum classifies them as Rh positive but still does not react with the rare Rh" cells.

The inheritance of the Rh types is conceived by Wiener as resulting from the distribution to the individual of one of eight allelic genes at a single corresponding locus in each of two paired chromosomes. The genes thus paired may be similar or dissimilar. The genes R^0 , R^1 , R^2 , R' , R'' , R^x , and R^z are dominant whereas r is recessive. The Rh types are designated as *phenotypes* because they can be demonstrated directly by the reactions of the cells with the three sera anti-Rh₀, anti-rh', and anti-rh". Each phenotype represents one or more *genotypes* which denote actual gene composition. The key to the recognition of some genotypes was found when Levine in the United States and Race and Taylor in England discovered sera containing agglutinins which reacted with an antigen which Levine named *Hr* (the letters Rh reversed to express the reciprocal relation to Rh antigens).

The theoretical reactions of the *genes* with the various antisera must be considered to demonstrate the reciprocal relation of *Hr* to Rh. This is shown in the accompanying table.

GENE-ANTISERUM REACTIONS

Genes	Antisera					
	Anti-Rh ₀	Anti-Hr ₀	Anti-rh'	Anti-Hr'	Anti-rh"	Anti-Hr"
r	—	+	—	+	—	+
R^0	+	—	—	+	—	+
R^1 ($R^{0'}$)	+	—	+	—	—	+
R^2 ($R^{0''}$)	+	—	—	+	+	—
r'	—	+	+	—	—	+
r''	—	+	—	+	+	—
R^x ($R' "$)	—	+	+	—	+	—
R^z ($R^{0' "}$)	+	—	+	—	+	—

If the same principles of nomenclature are extended to denote the presence of the *Hr* antigens, the genes H^0 , H' , and H'' might be included in the formulae of the genotypes, but this has not been considered advisable by Wiener, although some such implication is made.

A partial differentiation of the genotypes according to Wiener's theory is contained in the accompanying table.

An individual may receive the erythrocytes of another by transfusion or through the placenta of a fetus. In the latter case it is believed that the red cells of the fetus pass the placental barrier, possibly through minute leaks between the fetal and maternal circulation. When isosensitization has occurred by either route, isosensitivity is manifested in either of two ways. If the sensitized individual receives the antigen in transfused erythrocytes, intravascular hemolysis of the antigenic red cells results. Should the sensitized person be a woman bearing a fetus whose cells contain the antigen, the mother's antibodies diffuse through the placenta and cause various grades of hemolytic anemia in the fetus. The syndrome in the offspring is called *hemolytic disease of the newborn or erythroblastosis fetalis*.

Any of the isohemagglutinogens which are now recognized in human erythrocytes are possible antigens when introduced either by transfusion or pregnancy into the body of a person without them. Clinically it is apparent that some are much more antigenic than others. Some perspective may be obtained from an admittedly rough approximation in the accompanying table.

CLINICAL INCIDENCE OF ISOSENSITIZATION BY VARIOUS ANTIGENS

<i>Antigen</i>	<i>Given by Transfusion</i>	<i>From Fetus During Pregnancy</i>
A to A-negative persons	approximately 100%	rare
B to B-negative persons	approximately 100%	rare
M to M-negative persons	rare	unreported
N to N-negative persons	unreported	unreported
P to P-negative persons	rare	unreported
Rh to Rh-negative persons	approximately 5%	approximately 5%
Hr to Hr-negative persons	unreported	approximately 0.5%

Some elaboration of the statements in the table are desirable. When an A-negative person (belonging either to group B or O) receives an injection intravenously of erythrocytes containing the A substance, the titer of the natural anti-A agglutinins is greatly augmented in two or three weeks and it is several months before the initial titer is restored. The same phenomenon occurs when a B-negative person (either A or O) receives group B blood. This reaction occurs in nearly all instances and can be accepted as evidence of isosensitization. In striking contrast is the usual case of the B-negative mother who has a fetus of group A or AB (heterospecific pregnancy). In most instances no isosensitivity develops although there are now on record a few authenticated cases in which the A-negative mother has become sensitized to the A substance in the blood of the fetus. The natural anti-A agglutinins of the mother increase greatly in titer and apparently cause erythroblastosis in the fetus. The great difference between the antigenicity of the

Wiener's genotypes can be expressed in Fisher's terms as illustrated by the following examples: $\pi\pi = cde/cde$; $R^1r = CDe/cde$, $R^1R^2 = CDe/cDE$, etc.

An abstruse controversy has arisen as to the relative merits of the Wiener and Fisher theories of inheritance, part of which is symbolized by the two nomenclatures. It will probably be resolved by the accumulation of further data and the statistical analysis of gene frequencies to determine the validity of Wiener's hypothesis that the antigen combinations R^1 ($R^{0'}$), R^2 ($R^{0''}$), R^r ($R^{0'''}$), and R^z ($R^{0''''}$) are always inherited as gene units, as he contends, or are sometimes broken into their component parts, as the theory of Fisher implies.

Regardless of the genetic implications, it is probable that either nomenclature could be made to fit the facts as they emerge from further study. Both are logical and systematic. The utility of the two terminologies, however, is a highly practical problem. Wiener's has the advantage of greater verbal facility. The longest genotype is pronounced "R-two-prime, R-two-prime" whereas its equivalent is "Small-c-small-d-large-e, small-c-small-d-large-e" or, in a slightly shortened form, "c-d-large-e, c-d-large-e." On the other hand, while Fisher's symbols state all the facts, Wiener's imply much which is not directly expressed. There are no phenotype names in the Fisher terminology so that Wiener's terms must be employed for them. Some who wish to use the Fisher nomenclature employ incorrectly the formula for one gene as a phenotype name. Since only the sera anti-C, anti-D, and anti-E are readily available to most workers, there should be terms by which the results of tests with these reagents can be indicated without making unwarranted assumptions about the genotype. Wiener's phenotype names supply such a need. The reader is referred to the excellent book by Potter² for a detailed discussion of this subject.

Isosensitivity

One of the chief clinical implications of the studies on the Rh antigens has been the demonstration that human beings can produce antibodies against antigens in the erythrocytes of other persons. The phenomenon by which these develop is termed *isosensitization* (or *isoimmunization*) and the state is called *isosensitivity* (or *isoimmunity*). I prefer not to employ the term *immunity* because its primary meaning implies protection for the individual. Its use in this subject is very specialized and might lead to the erroneous conclusion that the antibodies which are developed are protective, whereas the opposite is true.

Acute and Chronic Anemias

Acute and chronic anemias are the prime indication for the transfusion of whole blood. Not only does the procedure supply circulating red cells which function as oxygen carriers for a time, but it has also been shown that the iron from the hemoglobin of the disintegrated cells is readily utilized in the formation of new erythrocytes if the hematopoietic system is competent.

Carbon Monoxide Hemoglobinemia and Methemoglobinemia

In carbon monoxide hemoglobinemia and methemoglobinemia new oxygen carriers are supplied by the transfusion of whole blood.

Immune Therapy

Many clinicians consider that transfusions of whole blood are of value in the treatment of systemic infections such as bacteremia, puerperal sepsis, rheumatic fever. The practice is purely empiric and is not based on well recognized immunologic knowledge. Evaluation of the efficacy of this type of therapy is extremely difficult.

Deficiency of Complement

It is well known that complement survives transfusion and even storage in blood for some time. There are few, if any, clinical conditions in which a deficiency of complement can be said to be of importance, however.

Deficiency of Prothrombin

There are few occasions when the necessity for correction of a deficiency in prothrombin is so urgent as to demand the relatively inefficient method of supplying it by blood transfusion. Much greater increases in prothrombin can be obtained in twenty-four hours by the administration of vitamin K. Occasionally, when there is severe hemorrhage because of hypoprothrombinemia, transfusions of whole blood may be indicated as an emergency measure in supplying preformed prothrombin.

Arresting the Action of Dicoumarin

When dicoumarin is being administered as an anticoagulant, its action may be superseded by supplying prothrombin in transfusions of whole blood, fresh or preserved.¹

Treatment of Leukopenia and Thrombopenia

Theoretically transfusion of blood is indicated for leukopenia and thrombopenia. Actually the number of leukocytes and blood platelets which can be

A and B substances, when given by transfusion and encountered in the blood of the fetus, has not been satisfactorily explained. There appears to be no distinction as to the route of contact in isosensitization to the Rh and Hr antigens.

Levine estimated that of 100 cases of erythroblastosis fetalis, about 90 are caused by sensitization to the Rh antigens, about six to the Hr antigens, and the remainder are due to the A, B, or unexplained antigens. Rh₀ is far more antigenic than the rest of the Rh group. Hr' is the most frequent of the Hr antigens, as a causative agent in sensitization.

In isosensitization to the Rh and Hr antigens acquired antibodies appear in the blood stream. There are at least two types. Those which produce the clumping of suitable erythrocytes, when the latter are suspended in saline solution, are variously termed *agglutinins*, *early immune antibodies*, *complete antibodies*, and *bivalent antibodies*. A second type characteristically causes the clumping of suitable erythrocytes only when no electrolyte has been added to the serum-cell mixture. These are called *blocking antibodies*, *late immune antibodies*, *incomplete antibodies*, and *monovalent antibodies*. Early in the course of isosensitization the agglutinins appear in the blood stream whereas the blocking antibodies occur later and their presence therefore denotes a greater degree of sensitization.

INDICATIONS AND CONTRAINDICATIONS FOR TRANSFUSION

The indications for whole blood transfusion have been based partly on sound practical considerations and partly on highly theoretical grounds which render the results doubtful.

Indications

Shock Due to Hemorrhage

It is now recognized that the best method of restoring the blood volume in shock due to hemorrhage is the transfusion of whole blood. Although transfusion of blood plasma or albumin is efficacious as a first-aid measure, it is distinctly inferior to the use of whole blood.

Shock with Hemoconcentration (Burns, Crush Syndrome)

Whole blood is inferior to the transfusion of plasma or serum albumin but is not contraindicated in cases of shock with hemoconcentration. It has been found that many patients with severe burns subsequently develop anemia which requires transfusions of whole blood.

		SERUM OR PLASMA (Agglutinins Indicated in Parentheses)			
		AB(α)	A(β)	B(α)	O($\alpha\beta$)
Cells	AB	—	+	+	+
	A	—	—	+	+
	B	—	+	—	+
	O	—	—	—	—

(+ indicates agglutination or hemolysis)
(— indicates no agglutination or hemolysis)

It will be noted that the plasma of group AB blood contains no agglutinins and therefore causes no reactions with cells from any other blood group. Group AB is therefore sometimes designated as the *universal recipient*. The erythrocytes of group O contain no agglutinogens and thus are not agglutinated by the plasma or serum from any other group. Group O is therefore called the *universal donor*, but can receive blood from no other group. A recipient belonging to group A can receive blood only from group O or group A donors. A group B recipient will tolerate blood only from group B or group O. The rare exceptions to these statements have been noted in the discussion of the subgroups of A and the irregular agglutinins.

It is also evident that the blood group of unknown cells may be identified by determining their reaction to the serum or plasma of groups A and B. These serums are therefore used for grouping. A slight additional check may also be obtained by the use of group O serum. When the unknown cells are found to be agglutinated by group O serum but by none of the others, it should call attention to errors in labeling or manipulation, or to deficiency in potency of the A or B serum.

Grouping Serums

Sources

The sources of grouping serums are at present: rabbit antiserums prepared commercially, human serums prepared in reputable laboratories from donors selected because of natural high titer of agglutinins, and human serums prepared by the worker who is to employ them.

Preparation

Human serum may be prepared from blood collected from donors selected because of high natural titer of agglutinins. Several methods of increasing the potency of human grouping serums have been introduced. Serums of low titer may be employed by precipitating with ammonium sulfate, washing, and redissolving the globulin fraction according to the method of Thalheimer.⁸

injected by transfusion is small, and one must question the efficacy of the procedure.

Treatment of Hemophilia

The coagulation time may be greatly diminished temporarily in hemophilia by the transfusion of whole blood.

Contraindications

Cardiac decompensation, either potential or actual, should serve as an almost absolute contraindication for blood transfusion. The question should always be raised before transfusion is performed of whether the patient's circulation will stand the increased load of the additional blood volume. This point is frequently overlooked when the need for transfusion is urgent. In certain situations it is a justifiable procedure to perform phlebotomy prior to transfusion.

BLOOD GROUPING AND CROSSMATCHING

Grave Responsibility of the Technician

It cannot be overemphasized that the determination of the blood groups and the crossmatching of bloods before transfusion impose a responsibility on the technician which is almost unique in clinical medicine. The usual laboratory test serves only as a supplement to the clinical diagnosis, and the clinician is frequently in a position to suspect or detect errors made in the laboratory. The patient is not placed in hazard of his life as a result of mistakes made in the usual laboratory procedures. The situation is completely reversed, however, in the tests preliminary to blood transfusion. An error may result directly in the death of a patient, and the clinician may be unable to detect the mistake in advance. The very nature of the tests always makes the possibility of errors imminent for the following reasons: (1) The technic of the tests is deceptively simple (2) There are no inherent absolute checks in the procedures. (3) A negative result, i.e., absence of agglutination, has the same diagnostic weight as a positive result. (4) The urgent requirement of the patient frequently places on the technician the psychological handicap of working against time.

Blood Group Interactions

The interactions of the blood groups must be known to determine the identity of groups and to practice transfusion. The chief reactions are tabulated as follows:

tion: One expresses the titer in terms of original dilution of the serum before the cell suspension is added, the other includes a correction for the volume of cell suspension and, possibly, saline solution added. A simple set of experiments will convince the reader that dilution of the serum-cell mixture within fairly wide limits will not alter the ability of the agglutinins to act upon the cells provided opportunity is given for the cells to come into contact with one another. It is therefore only proper to express the titer in terms of the original dilution of serum employed making no correction for added cell suspension or saline. Another obstacle in stating the numerical titer of a serum is that there is no adequate method of determining the sensitivity of the test cells which the worker may employ. A third variable is the method of reading the end point of agglutination in a series of dilutions of serum—whether to detect agglutination macroscopically or microscopically.

The author recommends the following method for the determination of dilution titer: Serial dilutions of serum in geometric progression are made in small serologic test tubes. A 2% cell suspension is made in the proportion of 1 drop of blood to 1 cc. of isotonic saline solution. Equal volumes of cell suspension are added to each tube of diluted serum. The tubes are then centrifuged at low speed for one to three minutes. The mixtures are shaken to resuspend the cells and are poured onto a glass plate. Agglutination is detected with the naked eye by illumination of the suspensions with a beam of light from below. The titer is expressed in terms of dilution of original serum without correction for added cell suspension. It is desirable to employ fresh cell suspensions from the same source for each titration.

The Subcommittee on Blood Substitutes of the National Research Council has accepted the following criteria for blood grouping serums:

Group A serum (anti-B) Minimal titer. Prepare a 1:16 dilution of the serum by mixing 0.1 cc. of serum with 1.5 cc. of saline. Mix one drop (0.05 cc.) of the diluted serum on a slide with a drop of a group B fresh cell suspension (2%). If possible, set up a parallel test with a cell suspension from a second individual of group B. Mix with an applicator or toothpick, agitate by rocking the slide to and fro at intervals of one minute. The titer of the serum is satisfactory if agglutination readily visible to the naked eye appears in less than 10 minutes with both bloods.

using
eye

group A cells. The 1:16 dilution should agglutinate A₂ cells in 10 minutes.

Speed and intensity of agglutination (avidity). Test as described for A serum except use group A cells at least one specimen of which is A₂. Distinct clumping should be visible within 60 seconds with A₂ cells and within 15 seconds with A₁ cells.

Specificity. Each serum should be employed to test at least 50 bloods taken at random

The blood serum may be concentrated by allowing the evaporation of water through a cellophane bag. The globulin fraction may be concentrated by precipitation in ethanol at low temperatures in vacuo.⁹ Witebsky¹⁰ has recently perfected a method of increasing the titer tremendously by repeatedly injecting the donor intravenously with solutions of the A and B substances. The autoclaved saliva of a secretor of appropriate group may be injected intramuscularly into a suitable donor (Wiener).

Liquid serums will keep for months in the refrigerator under aseptic conditions. Frozen or dried serums will retain potency for years. Fresh serums should be inactivated by heating in a water bath for sixty minutes at 56 C. to destroy the complement which might otherwise permit the action of isohemolysins which might be present. Serums stored in the liquid state for two weeks or more in the refrigerator are usually incapable of producing hemolysis. The serums may be colored with aniline dyes for identification, yellow for A and blue for B is recommended.

Rabbit anti-serums are prepared by repeated intravenous injections into rabbits of human red cells of the appropriate blood group. The animals are then bled and serum prepared. The anti-A serum should be absorbed by group B cells, and the anti-B should be absorbed by A cells to eliminate the species agglutinins. The serum is then dried and mixed with powdered sucrose. The mixture is packaged in a moisture-proof vial containing a dehydrating agent. Care must be employed not to expose the dry powder to the air unnecessarily, as it readily absorbs water and forms a cake. Animal immune sera agglutinate the cells of *all* infants, whether Rh positive or Rh negative. They have not proved very satisfactory.

Potency

Every writer stresses the importance of working with grouping serums of high potency, yet the most common cause of errors in the determination of blood groups by the inexperienced is the use of serums of inadequate strength. One reason for this has been the vagueness with which "adequate potency" has been defined. In the evaluation of typing serum, tests should be made for (a) specificity, (b) dilution titer, or greatest dilution causing agglutination, (c) avidity or speed of reaction. There is good correlation, for the most part, between the results obtained from tests (b) and (c), but some workers state that serums are occasionally encountered which agglutinate in high dilution but which react slowly in concentrated form. The difficulties met in defining potency exactly so that other workers may duplicate results should be briefly discussed. There are two methods of calculation of dilu-

group of recipient and prospective donor and to crossmatch the two bloods in five minutes when the centrifuge technic is employed throughout.

Agglutination may be detected either with the naked eye or through the microscope. When potent grouping serums are used, one method is as good as the other. The inexperienced observer has a tendency to rely on the microscope, but with a little practice he will find the macroscopic method as accurate and much faster. There are probably more errors made with the microscope in assigning diagnostic importance to insignificant clumps of cells. In crossmatching, microscopic readings are more necessary because the

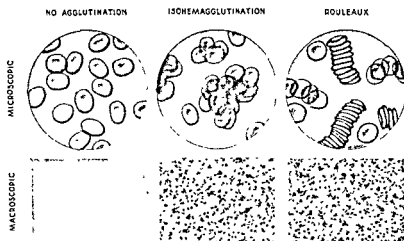


FIG 82 Appearance of serum-erythrocyte mixtures in tests for blood group and for compatibility

potency of the serums involved is frequently weak and the resulting cell clumps may be small. Rouleau formation may also occur and this is better differentiated from true agglutination by means of the microscope. In grouping it is usually practical to use both methods of reading, checking the suspensions which are apparently negative to the naked eye by the microscopic appearance. In mass blood grouping microscopic reading is impractical.

Slide Methods

On a microscope slide, appropriately labeled with a wax pencil, a drop of group A serum is placed on the left and a drop of B serum on the right. A drop of the red cell suspension to be tested is then placed on each drop of serum. The slide may then be allowed to stand at room temperature for

in parallel with a serum of known potency. At least one blood belonging to group A₂B should be included in the series

It is to be hoped that the problem of evaluation of typing serum will be still further clarified in the near future when some authoritative body will make available a standard reference serum with which products may be directly compared.¹¹ This will compensate for variations in sensitivity of test cells and in individual technics.

Methods of Grouping the ABO System

The identification of blood cells according to the ABO system consists in the addition of grouping serums from groups A (anti-B) and B (anti-A) to suitable specimens of erythrocytes to be tested. The presence or absence of agglutination of red cells is noted after an appropriate interval of time. The mechanism of agglutination operates in two steps. The union of agglutinin in the serum with specific agglutigen in the erythrocyte takes place at once. Most of the time consumed in the test is taken in bringing the red cells in contact so that clumping will occur. When the red cells are allowed to settle by gravity in a test tube through a depth of 3 mm., the time required for reading the test will be very much longer than when the red cells are in a drop on a glass slide and travel only a fraction of a millimeter to reach the bottom. Agitation of the cell mixture by tilting the slide to and fro will increase contacts between cells and accelerate the reaction. Centrifugation of the test tube containing the cell mixture will produce results in the shortest possible time. Comparative tests employing the same erythrocyte-serum mixture with the various methods will demonstrate that all are of equal accuracy if the proper time is allowed for each. Adequate time required for the different methods is as follows: suspension settling in a test tube by gravity, 120 minutes; cells settling in a drop on a slide, 30 minutes; cells agitated on a slide, 10 minutes; suspension centrifuged in a test tube, 1 minute. It is also true that the methods employing agitation of the cells tend to produce larger clumps of cells and are thus more easily read with the naked eye.

The choice of method, therefore, depends almost entirely on the time available in which to perform the test and the equipment at hand. Some authors have stressed as an advantage of plasma transfusions over the use of whole blood that the former saved much time by the elimination of grouping and crossmatching. I feel that this argument would be greatly weakened if faster methods of testing were adopted wherever practical. With the equipment of the modern hospital laboratory it is possible to determine the blood

temperature, never at 37° C., (c) contaminated serums, (d) panagglutination occurring in certain diseases, detected by testing cells against group AB serum, (e) autoagglutination, detected by testing cells against serum from same blood, (f) false agglutination of umbilical cord blood, (g) errors in labeling, (h) clotted blood.

In case of doubt in the identification of the agglutinogens of the cells, further check may be made by testing the serum against suspensions of cells known to belong to groups A, B, and O.

Blood Grouping en Masse

It is not generally appreciated that the determination of the blood groups of large numbers of specimens has special potentialities for errors when undertaken even by experienced workers. This is true for the following reasons: The opportunity for clerical error is greater, the chance of mislabeling multiplies with the large number of specimens, there is no immediate check-up by crossmatching such as occurs when a transfusion is to be performed as an immediate result of the laboratory procedures. Data have been presented by me elsewhere¹² on the typing of over 5000 blood specimens which showed that the errors made by individual workers in such situations varied from 1 to 10 per cent. It was recommended that all projects for grouping large numbers of bloods be carried out with two independent determinations on each blood. The results of the two tests should then be compared and the subjects of discrepancies be rechecked. This proves a much simpler and more reliable method than checking by grouping the serum from each blood. In the latter case, more time is consumed in the separation of the serum from the cells and one is dealing with serums of unknown titer which are frequently weak and difficult to evaluate.

Methods of grouping which are desirable when tests are performed prior to a transfusion and where speed is necessary, are impractical and unnecessary in mass typing. The centrifuge method is ideal for the transfusion service where each test is performed separately. In mass grouping the result of any one test is not immediately sought and the advantage of speed in the diagnosis of the individual blood is lost. Without special equipment, it takes more time to test 500 bloods with the centrifuge technic than by the slide method. The author recommends for mass agglutination the use of large glass plates upon which a number of tests may be performed more or less simultaneously. The serum and cell suspensions are allowed to remain quiet at room temperature for thirty minutes, after which the plate is agitated to

30 minutes, after which it is tilted several times to agitate the mixture. Suitable precautions against evaporation or depredation by insects may be necessary. The presence of agglutination may be observed with the naked eye, using a white background, or by reflecting light from below through the cell suspension. The preparation may also be examined in the microscope. To accelerate the test, the slide may be continuously tilted to and fro, by hand or mechanically, for ten minutes.

Another method employs whole blood without making a cell suspension. The skin of the subject is punctured; a drop of blood no larger than the head of a pin is placed on the slide, and a drop of typing serum is added immediately before clotting occurs. Continuous mixing is accomplished with an applicator or a toothpick. Some practice is required to obtain a sufficiently small drop of blood and to accomplish mixing before coagulation occurs. If strong typing serum is used, the reading time can be thirty seconds. This method requires the presence of the subject when the test is performed, and there is no cell suspension for subsequent checking.

Test Tube Methods.

Suitably labeled test tubes without lips measuring 7 to 13 mm. (inside diameter) by 70 to 100 mm long may be used. In a tube is placed a drop of serum, a drop of cell suspension, and a drop of isotonic saline solution. The tube may be allowed to stand at room temperature for 120 minutes or may be centrifuged for one minute at low speed. It is then shaken to resuspend the cells. Agglutination may be observed with the naked eye either by tilting the tube and looking through a thin layer of fluid illuminated by a beam of light or by pouring the suspension on a glass slide and examining as described under "Slide Methods." Some workers prefer to use a hand lens to examine the mixture in the tube.

Sources of Error

False negative reactions may be caused by (a) weak serums, (b) insensitive cells in infants, or in groups A_2 or A_2B , (c) insufficient time of observation, (d) use of excessively heavy cell suspensions which absorb agglutinins, (e) errors in labeling, (f) failure to inactivate hemolysins in fresh serums. False positive reactions may be due to (a) rouleau formation (usually differentiated microscopically by demonstration of stacks of cells, clumps are broken up by dilution of serum with saline and shaking), (b) cold agglutination due to agglutinins which react at low temperature, seldom at room

breaking up the sediment in the bottom of the tube, the borders of the cell mass may be inspected for the characteristic patterns in Figure 83.

The slide method of Diamond and Abelson⁶ has been widely used with satisfaction. This employs a 40 to 50% erythrocyte suspension in serum or plasma from the same source, in neutral serum from a person of group AB, or in serum albumin, either human or bovine. A drop of antiserum and cell

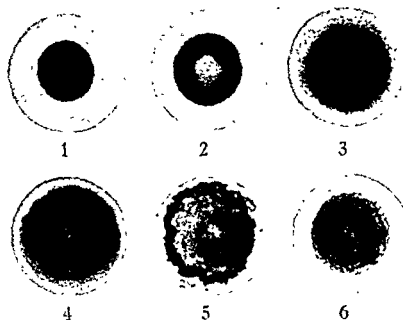


FIG. 83. Macroscopic appearance of sedimented erythrocytes in test tubes viewed from beneath in testing with anti-Rh serum (Landsteiner and Wiener, *J. Exper. Med.*)

suspension are mixed on a slide. The slide is held over an illuminated box which also furnishes the heat for incubation. The slide is frequently tilted and signs of agglutination are expected within three minutes, if the test is positive. Practice is required to differentiate rouleaux from true agglutination.

Titration of Acquired Antibodies in Isosensitization

Anti-Rh and anti-Hr agglutinins and blocking antibodies may be demonstrated in the blood serum of the person who has been sensitized to the antigens. Perhaps the simplest method is to set up two parallel titrations. Serial dilutions of the serum to be tested are made in isotonic saline solution in a series of serologic test tubes. A 2% suspension of test erythrocytes in

resuspend the cells and the tests are read. Specimens which are the subjects of discrepancies in the two independent determinations should be checked with serums from several different sources.

Typing in the Rh-Hr System

The determination of Rh types is employed in the transfusion laboratory for three purposes: (1) the selection of donors for blood transfusion, (2) the study of the mechanism of isosensitization in hemolytic disease of the newborn, (3) to determine whether the husband of a sensitized wife is homozygous or heterozygous.

Most commonly information is desired as to whether the prospective recipient and donor of a blood transfusion are Rh positive or Rh negative, so the Rh-negative recipients will be given Rh-negative blood to avoid isosensitization. For this purpose it is frequently advised that the cells of the donor be tested with anti-Rh₀' serum (the 87 per cent serum) which will prevent donors belonging to types rh' and rh'rh'' from being used for rh recipients. The blood of the recipients should be typed with anti-Rh₀ serum (the 85 per cent) to classify those belonging to types rh' and rh'rh'' as Rh negative so that they will not receive the Rh₀ antigen in transfusion.

To study the mechanism of isosensitization a detailed typing of the blood of the husband, wife, and living children is necessary. This requires the use of anti-Rh₀, anti-rh', and anti-rh'' sera.

When a family in which erythroblastosis has occurred desires an estimation on the possible outcome of future pregnancies, all members of the family should be typed with respect to the Rh system. In addition, if the husband belongs to type Rh₁ or rh', the agglutination of his cells by anti-Hr' serum will indicate in all probability that he belongs to the genotype R¹r or r'r and therefore 50 per cent of his offspring with an rh wife will be of the genotype rr and hence free from erythroblastosis fetalis.

Anti-Rh and anti-Hr antibodies differ from anti-A and anti-B agglutinins in two important respects, the serum-cell mixtures must be incubated at 37° C., and the agglutinates which are formed are very fragile and must be observed and handled with care.

Several procedures are employed in Rh typing. In the test tube method one drop of a 2% erythrocyte suspension in saline solution is placed in a serologic test tube with a drop of the specific antiserum. The serum-cell mixture is incubated at 37° C. in a water bath for thirty to sixty minutes. The tube is then centrifuged for one minute at low speed, the tube is then removed cautiously and tilted and rolled during inspection for agglutination. Before

Several points should be emphasized in connection with crossmatching in contradistinction to blood grouping. Crossmatching is generally performed when transfusion is imminent and speed is desired, so that the centrifuge is particularly recommended. Microscopic examination for agglutination is more necessary in crossmatching because the potency of the serums is unknown and may be very weak, and therefore the clumps of cells may be small. Thirdly, hemolysis should be looked for. The serums in the cross match are usually fresh and are not inactivated. The most dangerous situation is the occurrence of a high titer of hemolysin in the recipient. In the cross match the observer may miss the agglutination in the major match and, finding the clumps have disappeared, may conclude that the bloods are compatible, whereas the cells have agglutinated and the clumps have hemolyzed. I have known of at least two fatalities from this error.

Crossmatching to detect the anti-Rh factor is performed with a technic precisely similar to that for the determination of the Rh factor except that the cells of the prospective donor and the serum of the recipient are combined.

SELECTION OF THE DONOR

Qualifications

It is common practice to accept as blood donors persons of either sex between the ages of 18 and 50 or 60 years. They should state, preferably in writing, that they are in good health. A history of malaria at any time should be cause for rejection. If the prospective donor is having symptoms of pollen hay fever or asthma, he should not be used although donors with hay fever are generally acceptable during seasons when pollen is not present in the air. A history of angioneurotic edema or chronic urticaria should be cause for rejection. A story of chancre, gonorrhea, or other venereal disease should lead to closer scrutiny of the question as to whether the person is suitable as a blood donor. The person who gives a history of jaundice within six months should be rejected on the possibility that it was of the infectious type. It has been recently learned that the virus of this disease is transmissible by transfusion and that the incubation period is from 30 to 200 days. The work of Oliphant¹³ showed that the blood of an infected person is not infective after two and one-half months, but the virus may be transmitted before jaundice has appeared in the donor. The person with the initial symptoms of an upper respiratory infection should not be accepted because subsequent

saline is added to each tube in the series. In the other row of tubes the serial dilutions of serum are made with neutral serum or serum albumin, either human or bovine, without added sodium chloride. The same test erythrocytes are employed but they are suspended in the protein solution instead of saline. All the cell-serum mixtures are then incubated for 60 minutes at 37° C. and evidence of agglutination observed. The greatest dilution in which clumping occurs in the saline series is the titer of the agglutinins, whereas the reading in the tubes without added saline is the titer of the blocking antibodies.

Determining the Subgroups of A and AB

If determination of the subgroups of A and B is desired, special serum is required. The usual anti-A grouping serum contains both anti-A₁ and anti-A₂ agglutinins. If it is sufficiently potent, all the subgroups of A agglutino-gen will be agglutinated by it, the A₂ and A₂B reacting less strongly than the others. The anti-A serum may be altered by adding one-fifth the volume of known A₂ cells, mixing, and allowing to stand for sixty minutes. The cells absorb the anti-A₂ agglutinin and are then separated from the serum, which is then known as "absorbed anti-A serum." This will agglutinate only A₁ and A₁B cells. The A₂ and A₂B cells are identified by failure to react to this serum.

CROSSMATCHING OF BLOOD FOR TRANSFUSION

Whenever possible, crossmatching of the blood of the recipient with that of the prospective donor should be performed prior to transfusion, even though homologous groups are thought to be involved. Statistics as to the low incidence of transfusion reactions without tests of compatibility will not be impressive to the worker who has crossmatched many bloods and encountered occasional incompatibilities, particularly in groups A and AB. Crossmatching is also a check on the blood grouping. In matching infants' blood and the groups A₂ and A₂B, it may occasionally be found that the agglutinins in the serum of the blood tested are stronger than those of the typing serums and will thus reveal errors in the grouping.

The procedures for crossmatching are the same as for blood grouping, but the sources of cells and serums are different. The cells of the donor and the serum from the recipient are combined in what is termed the "major match", the combination of the recipient's cells and the donor's serum is known as the "minor match." The major match is of prime importance to the transfusion, but performance of the minor match is an additional check on the correctness of the group determination and should be carried out whenever possible.

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events might prove that he was suffering from the prodromes of contagious disease or of pneumonia. A recent toxic manifestation from sulfonamides should be cause for rejection if the recipient is to receive similar drugs. I have seen one example of passive transfer of sensitivity to sulfathiazole by transfusion.

The Blood Donor Service of the American Red Cross has established the value of 12.3 Gm. per 100 cc. as the minimal level of hemoglobin for an acceptable blood donor, male or female. Determination of acceptability, using this criterion, is made by puncturing the finger of the prospective donor and collecting several drops of blood in a glass capillary tube. The blood is quickly expelled by means of a small rubber bulb (used for vaccine virus) into a bottle containing a solution of copper sulfate with a specific gravity of exactly 1.053. A globule is formed by precipitation of protein on the surface of the drop. If the drop floats temporarily, the hemoglobin is less than 12.3 Gm. and the prospective donor is rejected. If the globule sinks, the hemoglobin is greater than 12.3 Gm. and the donor is accepted. This method is an adaptation of the procedure for determining hemoglobin devised by Phillips *et al.*¹⁴

Blood Group of the Donor

It is possible to dispense with the determination of the blood group of the donor in an extreme emergency where a compatibility test can be performed. In general, it will be found that this practice wastes more time than it saves. The identification of the blood group of the recipient and of prospective donors usually enables the prompt selection of suitable blood for transfusion. The use of blood of homologous groups for transfusion is usually recommended, but this practice in itself does not necessarily insure compatibility, and crossmatching should be performed. In case the recipient belongs to group AB or B, selection of a homologous group from a small number of persons may be impossible. A recipient of group AB can take blood from any other group but a group B person must receive blood from his own group or from group O. The possible implications of these cross transfusions will be discussed in consideration of the "universal donor."

The Universal Donor

By definition group O blood contains the two agglutinins anti-A and anti-B in the plasma but no agglutinogens in the cells. Persons of this group were called "universal donors" on the assumption that their agglutinins would be

rendered ineffective when diluted with the plasma of a recipient of a heterologous group. The absence of clinical reactions after transfusions employing the universal donor in which the titer of the transfused incompatible agglutinins is relatively high has led to the realization that at least two other factors are at work to inactivate the heterologous agglutinins: combination with small amounts of group-specific substance in the plasma of the recipient and, more important, combination with group-specific substance in the fixed tissues of the recipient. These facts are recognized by all, and it is agreed that blood from most group O donors is safe when transfused into recipients of heterologous groups. The use of universal donors has been in vogue for many years in some large hospitals in this country and has re-

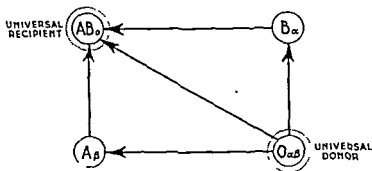


Fig. 84 Compatible blood groups for transfusion

ceived trials on a large scale in many of the armies engaged in World War II. A controversy still exists as to whether the group O blood with an exceptionally high titer of agglutinins is safe for transfusion into other groups. The Rh factor was discovered in 1940 so that cases reported before that time purporting to show reactions from universal donor blood included no examinations for the anti-Rh factor. Aubert *et al.*¹⁵ have injected large amounts of plasma selected for agglutinins of high titer into recipients of heterologous groups and have shown minor amounts of agglutination of the recipients' cells, but there were no important clinical reactions. Klendshoj *et al.*¹⁶ have reported one case in which group O blood apparently caused a fatal hemolytic reaction in a recipient when the presence of the anti-Rh factor had been excluded. Further study is required to settle this point. If the agglutinins in group O blood are deemed dangerous in transfusion, there is now at hand a means of inactivating them *in vitro* by the addition of a solution of the A and B group-specific substances described by Witebsky *et al.*

COLLECTION OF BLOOD FOR TRANSFUSION

General Care of the Donor

Frequently, since blood transfusion has become a common procedure, the donor is an inexperienced person who approaches the operation of giving blood with some trepidation. If he can be in the company of others who are undergoing the same procedure without discomfort, the example is often reassuring. A long period of waiting before drawing the blood, especially when the time is spent in conversation with others who are also apprehensive, is a poor psychological prelude. Care taken to provide a cheerful and reassuring environment for the collection of blood will repay the effort many times in lessening the number of donor reactions.

The donor is placed in the recumbent position on a table, and the collecting flask is so arranged as to be out of his sight. The antecubital fossa of the extended arm is prepared with tincture of iodine and alcohol. A tourniquet is placed around the arm just above the prepared site. A convenient method is the use of the cuff of a sphygmomanometer, inflated to a pressure of about 40 mm. of mercury. With a 24 gage needle, a bleb of 2% solution of procaine is raised over the vein to be entered. The vein selected is usually the largest and most accessible one in either arm. After local anesthesia has been attained, a needle of 13 to 16 gage is inserted in the vein through the procaine wheal. The blood is then allowed to run freely, either by gravity or by the application of gentle suction. In many donors it will be found possible to release the tourniquet entirely without impairing the rate of flow. Other donors may have to flex and extend the fingers slowly to secure an auxiliary pumping action. It is helpful in such a case to give the donor something to grip, such as a roller bandage or a piece of sponge rubber.

When sufficient blood has been withdrawn (usually not over 500 cc.), the tourniquet is loosened, the donor's hand is relaxed, a pressure bandage is placed over the site of injection, and the needle is quickly withdrawn. The donor is asked to flex the arm, and the bandage is later fixed tightly in place for several hours. The donor is requested to lie in the supine position for fifteen to thirty minutes. The foot of the table may be elevated slightly. At the end of the rest period the donor may be allowed to sit up cautiously and finally to stand. An experienced person should be present and on the alert for signs of vasomotor disturbance and, at the first indication, should tactfully suggest that the donor lie down.

When the donor feels able to walk, he should be encouraged to drink fluids and may be given light refreshments such as milk drinks, cookies, or crackers.

Excessively hot or cold drinks should be avoided. The ingestion of hot drinks by a person who has recently lost blood may further deplete the circulating blood volume sufficiently to cause syncope.

Reactions in Donors

It was formerly thought that practically anyone could lose 500 cc. of blood without exhibiting symptoms. With the recent experience in bleeding thousands of persons it has been found that from 1 to 5 per cent of donors have more or less severe symptoms. The manifestations vary from a feeling of weakness on resuming the erect position to syncope. The symptoms may appear at any time during the collection of blood or up to an hour or so after the donation. There is usually a sudden pallor and profuse sweating; the blood pressure falls and the pulse is slow. Consciousness may be completely lost. Vomiting, involuntary defecation, and micturition may occur.

The most disconcerting and dangerous type of reaction is the delayed faint. The donor may feel and appear perfectly normal at the conclusion of the usual rest period following donation. He then walks away from the bleeding center and suddenly faints some time later, at peril to himself. Fortunately such instances are rare, but no method is now known of preventing this type of accident. The causes of fainting in donors are not fully understood. Extensive studies have been made with inconclusive results. Obviously some fainting is due to apprehension in the inexperienced individual. This type is contagious. The excited conversation of apprehensive persons awaiting their turns to give blood is not reassuring to the novice. A few would-be donors swoon before they are ever touched with a needle. A psychogenic etiology does not seem to explain the case of the person who is not afraid and in whom the symptoms only appear after most or all of the blood has been withdrawn. There are cases of experienced donors who always faint when a certain definite amount of blood has been collected. It has been suggested that these persons have sensitive carotid sinus reflexes.¹⁷

A rare type of reaction is the occurrence of generalized convulsions with or without loss of consciousness. The clinical picture resembles an epileptiform attack. It has been shown that many persons who react in this way have the pattern of the epileptic in the electroencephalogram although they have never had clinical epilepsy.

Hyperventilation tetany occasionally occurs in an apprehensive blood donor. I have seen at least one case in which hyperventilation was not evident as a cause for the tetany, and neither holding the breath nor rebreathing would relieve the symptoms.

TECHNIC OF INJECTION OF BLOOD

Methods

Modern experience has left little, if any, argument in favor of the direct methods of transfusion. They are inconvenient, considerable skill and complicated apparatus are required, and the propinquity of the donor and recipient is frequently undesirable. As knowledge of pyrogens has increased, less has been heard about the toxicity of sodium citrate. Recent experience with massive transfusions of citrated blood and plasma has conclusively demonstrated that the amount of sodium citrate injected is not harmful and does not produce reactions.

A detailed description of the hundreds of methods of indirect transfusion would be unprofitable. It is trite to say that successful transfusion depends upon introducing the blood into the circulation of the recipient, nevertheless, many transfusions fail because this cannot be accomplished. I know of no way of remedying this situation except by practice on patients. Although most physicians assume an air of superiority toward the operation of transfusion apparatus, it is surprising how many are not familiar with some of the laws of hydraulics upon which the successful operation of the equipment depends. It is splendid advice, seldom followed, that the physician should practice with a dummy apparatus before being confronted with a flask of blood which is destined for the vein of his patient.

It is generally recognized that citrated blood should be passed through some type of filter before administration. Obviously the filter should be sufficiently fine to withhold particles of fibrin and debris which would otherwise clog the needle. There is no agreement on the minimum size of the pores of the filter. The problem of filtration is not serious when fresh blood is being given, but when preserved blood is employed, filters of fine mesh which are satisfactory for the former frequently become completely plugged with the shreds of fibrin and degenerated leukocytes which accumulate during storage. The filtering surface must be greatly enlarged or the mesh must be much coarser to pass preserved blood. This problem has not been satisfactorily solved for the administration of preserved blood in a "closed" system. In an "open" system the blood may be poured through three or four layers of sterile surgical gauze with satisfactory results.

Selection of a Site of Injection

In general, any accessible vein which is sufficiently large can be used for transfusion. One route frequently ignored when the peripheral vessels are

destroyed is the femoral vein. The physician should become familiar with this technic for use in an emergency. This region of the body is seldom traumatized, and knowledge of the method of administration may be life-saving. The femoral artery is palpated one inch below the inguinal ligament; the femoral vein will lie just medial to it although it cannot be seen or felt. The needle is inserted blindly and perpendicularly at this site until venous blood is withdrawn. No fixation of the needle is usually required although it appears to be in a precarious position.

I have had no experience with intrasternal transfusion. Three cases in which fatalities have occurred are known to me. In each instance the needle pierced the inner table of the sternum and blood was injected into the mediastinum. It is a matter of opinion as to whether this could have been avoided by skilful manipulation. It is to be hoped that a needle will be devised which will make impossible such accidents.

Dosage

It is usually stated that the maximum dose of blood for children is 20 cc. per kilogram of body weight. This value has been satisfactory. The usual volume of blood given to an adult in one transfusion has been 500 cc. This has been determined largely by the limitation of the donor and not by the needs of the recipient. There is much more reason for adhering to this practice when fresh blood transfusions are employed than when a large reserve of stored blood is available. In determining the volume of blood to be administered at a single transfusion two factors must be considered: the circulating blood volume of the recipient and the condition of the cardiovascular system. With large quantities of blood at hand in a bank, it is logical and practical to replace the blood lost by acute hemorrhage, even though the volume is several liters. This is the accepted treatment for shock from hemorrhage. If the cardiac function is impaired, even 200 cc. of added blood may result in frank decompensation. In the treatment of carbon monoxide poisoning, for example, when it may be necessary to transfuse large quantities of blood into a patient with heart disease, it is logical to bleed the patient before transfusion.

Rate of Injection

It is frequently stated that the maximum rate at which blood transfusions should be given is 20 cc. per minute. This limitation is misleading. In the treatment of severe grades of shock it has been found necessary to restore the circulating blood volume as rapidly as possible. The British have found

that a rate of 50 cc. per minute is desirable at times.¹⁸ This is practically unattainable in a gravity system unless a needle of 16 gage is employed. In lieu of this, the blood may be forced in under pressures greater than gravity. On the other hand, when cardiac function is impaired, many advise a very slow rate of transfusion. This practice has virtue when infusions of crystalloid solutions are being administered because the velocity of administration may be made to approximate the rate of loss of fluids from the circulation. It is doubtful whether this equilibrium can be obtained, however, when blood is being introduced into the circulation. Here one is adding a mixture of cells and protein solutions which are not intended to leave the circulation. The readjustments by loss of water into the tissues occur much more slowly after blood transfusion than was formerly thought.

Temperature

Formerly it was considered necessary to adjust crystalloid solutions and blood to body temperature before injecting them into the veins of the patient. There are few types of apparatus which will actually accomplish this. Most of the reactions attributed to the infusion of cold solutions have probably been due to the presence of pyrogens in the fluids. Pyrogen-free fluids or blood may be administered intravenously at temperatures as low as 10 C without reaction.¹⁹ Attempts at heating blood during transfusion may actually be disastrous if the temperature inadvertently becomes excessive. Much experience has proved that the heating of solutions and blood for intravenous administration is not only time-consuming and expensive but unnecessary.

SURVIVAL OF TRANSFUSED ERYTHROCYTES

Many recent studies have been made of the survival time of transfused erythrocytes to evaluate the various methods of preservation of blood. The methods of experimentation which have proved the most satisfactory are modifications of the agglutination technic first described by Ashby and the labeling of hemoglobin in the transfused erythrocytes with radioactive isotopes of iron. For details of the modified Ashby technic the reader is referred to the papers of Mollison and Young²⁰ and of Denstedt, Osborne, Stansfield, and Rochlin²¹ in which extensive studies are reported. With transfusions of fresh blood a variable number, up to 40 per cent, seem to disappear from the circulation in the first three days in some recipients. Thereafter there is an irregular decline in the number of transfused red

cells although some are retained as long as 120 days. When blood preserved as long as 15 to 20 days is transfused, the red cells seem to persist nearly as well as fresh blood if correction is made for the fact that all of the cells in the stored blood are 20 days older. Gibson and Evans,²² also Ross,²³ by labeling cells with hemoglobin containing radioactive isotopes of iron, have shown that when the transfused cells disintegrate in the recipient's circulation, the hemoglobin thus released is readily assimilated in the formation of new cells.

REACTIONS FROM BLOOD TRANSFUSIONS

The average physician is inclined to regard all reactions from blood transfusions as a single clinical entity. This is as meaningless and as unscientific as to be content with a diagnosis of "fever" in a patient whose body temperature is elevated. A variety of syndromes may accompany the transfusion of blood, and proper understanding of the etiology, prognosis, treatment, and prophylaxis can be forthcoming only by making a differential diagnosis.

Pyrogenic Reactions

Etiology

The work of Seibert in 1923 demonstrated that certain river bacteria, many of them nonpathogenic, can grow in distilled water and produce substances which cause fever when injected parenterally into animals. She named these substances "pyrogens" and showed that they pass through bacterial filters and their fever-producing ability is not destroyed by the usual amount of heat employed in the sterilization of fluids for parenteral use. When fluids containing pyrogens are administered parenterally to man, or equipment prepared with pyrogenic fluids is employed in parenteral therapy, a definite clinical syndrome is produced. There is great individual variation in susceptibility to pyrogens so that when a number of patients receive pyrogen-containing fluids from the same lot, not all, or even a majority, of patients may have reactions. Also involved are the factors of dosage and rate of administration.

Pyrogens may occur in the manufacture of crystalloid solutions for parenteral injection. They may also contaminate the equipment employed for the administration of crystalloid solutions or blood transfusions. Organisms may be introduced into the blood at the time of collection, and, during storage, pyrogens may be formed before the bacteria are killed by the nat-

ural bactericidal agents of the blood. The incidence of pyrogenic reactions in one series of 2423 blood transfusions was 2.1 per cent.²⁴

Clinical Description

The manifestations of this type of reaction occur during, or within an hour after, the parenteral administration of crystalloid solutions, blood, or plasma. The mildest type is characterized by the occurrence of a chill without fever. More violent reactions begin with chills and are accompanied by rapid rises in body temperature to levels from 100 to 105 F. During the height of the reaction the patient may be somewhat dyspneic and extremely uncomfortable. The fever persists for only a few hours after which the temperature resumes the preinjection level. There are no sequelae.

Diagnosis

The association with parenteral injections is prima-facie evidence, but other conditions must be considered. It is sometimes difficult, if not impossible, to differentiate a pyrogenic reaction from the clinical manifestation of the primary disease for which the patient is being treated. The onset of severe pyrogenic reactions resembles the initial symptoms due to intravascular hemolysis. The latter condition must be excluded with all possible speed by withdrawing a small amount of blood from the vein of the patient, centrifuging it, and inspecting the supernatant serum for free hemoglobin or bilirubin.

Treatment

The transfusion is discontinued. Usually no other measures are required. Sedatives may be given if the recipient is uncomfortable.

Prognosis

This type of reaction is annoying but usually not dangerous.

Prophylaxis

The prevention consists in scrupulous preparation of equipment and fluids to exclude the presence of pyrogens. The collection of blood should be performed preferably in a "closed system" with due regard for asepsis. If the equipment is thought to be contaminated with pyrogens but pyrogen-free crystalloid solutions are available, the equipment may be washed out with 100 to 200 cc. of sterile pyrogen-free fluid prior to use.

Urticaria

Etiology

About 1 per cent of blood transfusions is accompanied by urticaria of greater or less degree. Injection of plasma may also produce this reaction. One physician told me that he has developed urticaria several times following the intravenous administration of isotonic saline solution. In most cases of urticaria neither donor nor recipient give any previous history of urticaria, hay fever, or asthma. On the other hand, I know of one person whose blood has given urticaria to eight out of ten recipients receiving it. The donor has had many attacks of angioneurotic edema, some of which are definitely related to emotional disturbances. Some recipients developing urticaria after transfusion will fail to react the next day from transfusion of more blood from the same flask. Blood from the same flask has been transfused into two recipients, producing urticaria in one and not in the other. This type of reaction involves no known association with isoimmunity.

Clinical Description

During or soon after the transfusion of blood or plasma the patient develops urticaria. Only a few wheals may appear, or massive angioneurotic edema may develop. The eruption usually disappears spontaneously in a few hours.

Diagnosis

Diagnosis is made by inspection. The disease can scarcely be confused with anything else.

Treatment

The transfusion should be discontinued if the extent of the lesions is great or if edema develops in sites which are particularly vital, such as the pharynx or the larynx. Epinephrine hydrochloride (0.3 to 1 cc. of a 1:1000 solution) may be given hypodermically and repeated in a few minutes if the reaction proves severe.

Prognosis

There is very little danger from this type of reaction provided prompt treatment is given before edema of the larynx develops.

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Treatment

The therapeutic test and the emergency treatment is to place tourniquets on all four extremities. The tourniquets should be applied as near to the trunk as possible and only tightly enough to produce venous stasis. The arterial blood supply should not be shut off. Ebert and Stead²⁶ have shown that this maneuver results in the pooling of at least 15 per cent of circulating blood in the extremities. Prompt relief of dyspnea and disappearance of rales in the lungs should follow. About every twenty minutes the tourniquets should be released and reapplied, one at a time, to restore the circulation in the limbs. If blood or plasma has been administered, it is probably wise to withdraw 500 cc. of blood from the recipient after the efficacy of the tourniquets has been proved. If crystalloid solutions have been given, it is possible to temporize with tourniquets until the fluid has left the circulation. *Prompt diagnosis and treatment are sometimes lifesaving measures.*

Prognosis

The author has seen one adult who was not previously in cardiac failure die from left-sided heart failure from the injection of 200 cc. of compatible blood before the treatment for this condition was known. Another patient died in similar manner from 500 cc. of blood. Prompt recovery from the condition has been observed on many occasions when treatment was instituted.

Prophylaxis

The condition can frequently be prevented if the status of the cardiovascular system is considered more often when transfusion is contemplated. When a blood transfusion is indicated, the question should always be asked, Can the circulation tolerate the increased blood volume? In selected cases it may be possible to bleed the patient before administering a transfusion of blood or plasma. Suspensions of erythrocytes may be given for the treatment of anemia where the plasma in whole blood is deemed unnecessary and the increased volume of circulating plasma protein is dangerous.

Hemolytic Reactions

Etiology

The products of disintegrated erythrocytes are potentially dangerous when introduced into the circulation in sufficient quantities. There are two mechanisms by which this occurs. Extravascular hemolysis may be caused

Prophylaxis

Lack of knowledge prevents any effectual prophylactic measures. Prospective donors should be rejected if they give a history of chronic urticaria or angioneurotic edema. This precaution will prevent only a small number of reactions, however.

Circulatory Overload

Etiology

The normal heart can cope with a moderate increase in the circulating blood volume, but it has been shown by Murphy *et al.*²⁷ that when there is borderline compensation, the intravenous injection of as little as 500 cc of isotonic saline solution is sufficient to induce frank cardiac failure. Such a procedure is less than equivalent to the administration of a similar volume of blood or plasma as crystalloid solutions rapidly leave the blood stream and the resulting increase in circulating volume is therefore quite transient. On the other hand, the increase in circulating fluid obtained after the injection of blood or plasma is much more stable and therefore more dangerous to the circulatory system, which is already impaired. When circulatory failure is induced by the intravenous injection of fluids or blood, the left side of the heart fails first, and edema of the lungs results. Fatal left-sided heart failure has been observed by the author after the transfusion of as little as 200 cc of blood into an adult in whom there was previously no clinical sign of cardiac insufficiency. The condition should be anticipated when transfusions or intravenous infusions are administered to any patient who has heart disease or conditions predisposing to heart disease.

Clinical Description

During or within two hours after blood transfusion the patient may suddenly become dyspneic and extremely cyanotic. The heart rate is very fast and arrhythmias such as auricular fibrillation or auricular flutter may develop. The lungs are filled with coarse, moist, sibilant rales. Death may occur within one-half hour to six hours if prompt treatment is not instituted.

Diagnosis

Diagnosis depends on the appearance of the symptoms and signs and is proved by the response to therapy. When diagnosis is doubtful, the patient should be treated for the condition.

is usually "direct" if obtained at this stage, turning to "indirect" the next day. The jaundice fades within two or three days. The icteric phase may occur without the preceding acute phase.

The anuric phase, if it occurs, usually intervenes shortly after the acute phase. There may be complete anuria or oliguria with a daily excretion of 100 to 500 cc of urine. Hemoglobin is only present in the urine for the first two days at most. Casts of hemoglobin pigment can be observed in the urine for another day or so. When hemoglobinuria occurs, there is usually sufficient pigment to color the urine deep red or brown to black. During the anuric stage the patient may appear to be recovering except for the steady increase in the degree of azotemia, the daily urea nitrogen values of the blood frequently increasing almost by arithmetical progression.

Death from renal insufficiency seldom occurs before the fourth or after the twelfth day. The blood pressure remains normal in most cases although there are well authenticated instances in which hypertension developed. Convulsions are usually not a prominent feature. Coma supervenes during the last days of life. Spontaneous and complete recovery may occur at any of the three phases without development of the succeeding stages. In the anuric phase, diuresis may occur suddenly either as the result of treatment or spontaneously, and the evidence of nitrogen retention gradually disappears.

Diagnosis

The problem of diagnosis varies somewhat depending upon the phase at which the question arises. The acute phase must be differentiated from severe pyrogenic reactions and from circulatory embarrassment. The absence of rales in the chest excludes edema of the lungs. A sample of blood should be immediately collected in a clean, dry syringe and centrifuged in a clean tube. Simple inspection of the supernatant serum will reveal the presence of significant quantities of free hemoglobin or bilirubin. The icteric phase must be differentiated from jaundice due to other causes. If the icterus is accompanied by hemoglobinuria, the presumption of a hemolytic reaction can usually be made. The anuric phase of a hemolytic transfusion reaction must be distinguished from anuria due to shock, the crush syndrome, sulfonamide intoxication, quinine idiosyncrasy, blackwater fever, or nephritis. Differentiation may be impossible either clinically or pathologically in some cases.

If a hemolytic transfusion reaction can be diagnosed, a subsidiary problem is the cause of the hemolysis. Intravascular hemolysis is diagnosed by demonstrating incompatibility in the transfused blood. For this purpose, a

by the transfusion of whole blood which has undergone spontaneous hemolysis during storage; by the application of excessive heat or cold to the blood before transfusion; by the addition of large quantities of crystalloid solutions to whole blood in concentrations either hypertonic or hypotonic, by the addition of other hemolytic agents to blood which is to be transfused. Most of the conditions implied in this category are more frequently encountered in transfusions of preserved blood than when fresh blood is employed. Intravascular hemolysis may occur from the transfusion of erythrocytes containing agglutinogens which are incompatible with agglutinins occurring in the blood of the recipient. It may also occur from the transfusion of red blood cells the contents of which have been rendered hypertonic or hypotonic to normal blood plasma by improper preservation

At present, the preponderance of evidence supports the view that free hemoglobin itself in sufficient amounts is deleterious when it appears in the circulation in large quantities. When the plasma hemoglobin level exceeds a value between 35 and 45 mg. per 100 cc., hemoglobinuria occurs. The free hemoglobin circulates in the blood stream as such only a few hours, being rapidly converted to bilirubin in man. Some hemoglobin also forms a more or less stable combination with plasma albumin which has been designated as methemalbumin by Fairley. Much of the hemoglobin is rapidly taken up by the reticulo-endothelium

Clinical Description

For descriptive purposes it is convenient to divide the clinical course of a hemolytic transfusion reaction into several stages. The acute phase lasts from the time hemolyzed blood is introduced into the circulation until approximately one day post transfusion. If the total free hemoglobin released is small or if the amount introduced per minute is minimal, no immediate symptoms may occur. If the dose is larger, the patient experiences a moderate or severe chill followed by a transient fever. There may be dyspnea with a sense of constriction in the chest, cyanosis, and severe pains in the abdomen and in the lumbar region, radiating down the legs. If the initial symptoms are severe, shock may intervene promptly, and death results at this stage. I have observed an adult who died in shock within six hours after the transfusion of 200 cc. of incompatible blood. The acute phase is seldom prolonged beyond eight to twelve hours, after which the patient is relatively comfortable.

About six hours post transfusion icterus may develop. This is symptomless and is presumably hemolytic in type although the van den Bergh reaction

Unknown factors in the body of the recipient undoubtedly play an important role as some tolerate severe degrees of hemoglobinuria without renal failure whereas others develop fatal anuria. Death may result in the acute phase from shock or in the anuric phase from renal insufficiency. I have observed ten patients with transfusion anuria, seven of whom died and three recovered. Practically all had received some of the methods of therapy mentioned. I still have no definite opinion as to the efficacy of treatment.

Prophylaxis

The prevention of both in vitro and in vivo hemolysis depends on the care and skill with which the entire procedure of blood transfusion is handled. A blood transfusion service with trained personnel to supervise every detail has proved the best method of prophylaxis. As to the preparation of the patient, the administration of alkalis, to insure an alkaline reaction to the urine, prior to transfusion may be of some value in preventing anuria.

Transmission of Infectious Disease by Transfusion

Etiology

The principal diseases known to be transmitted by blood transfusion are syphilis, malaria, and infectious hepatitis. Theoretically almost any organism producing bacteremia in the donor could be transmitted to the recipient by injection. The transfer of brucellosis should be guarded against. It should be emphasized that the donor with a primary lesion of syphilis and a negative blood Wassermann reaction is potentially more dangerous in the transmission of syphilis than one with well established serologic evidence of syphilis. Malaria is notoriously a chronic disease, and the use of a donor known to have had it at any time is potentially dangerous. The long incubation period of infectious hepatitis of from 60 to 120 days is particularly deceptive. Transfusion syphilis differs from the usual type in that the first manifestation is the appearance of secondary lesions, the chancre being absent. The incubation period may be several months.

Diagnosis, treatment, and prognosis are problems in general medicine.

Prophylaxis

Obtaining a careful history and performing a physical examination should exclude most of the chances of transmitting disease by transfusion. Donors are rejected who have had jaundice within six months if the cause of the

sample of the blood to be given should always be held in reserve. If preserved blood has been given, osmotic hemolysis may be tested for by diluting the blood mixture with four or five volumes of 0.9% sodium chloride solution, centrifuging, and comparing the color of the supernatant with that of centrifuged citrated plasma from the mixture. A similar test may be employed using the recipient's serum or plasma instead of salt solution. If the hemolysis occurs under the conditions of the test, it is a fair assumption that the improperly preserved cells hemolyzed in the body of the recipient. Extravascular hemolysis may be presumed from a careful check of the history of the handling of the blood during the transfusion or demonstration of excessive hemolysis in the reserve specimen of blood.

Treatment

If the reaction is detected during the transfusion, the injection should be immediately discontinued. It has been stated that the transfusion of less than 200 cc. of incompatible blood has never been fatal in an adult. I have observed one patient who died from approximately that amount. In the absence of a certain knowledge as to the mechanism of transfusion anuria, it is probably safer to take immediate steps to insure an alkaline reaction in the urine either by administration of sodium bicarbonate or sodium citrate in large doses orally or by the intravenous injection of $1/3$ or $1/6$ molar sodium *r*-lactate solution. If the reaction is first detected during the icteric phase and there is no diminution in renal excretion, the recipient may be given alkalis by mouth and merely observed for possible development of oliguria or anuria. If the anuric phase develops, fluids should be given by mouth and saline and dextrose should be injected intravenously. In view of the uncertain prognosis without treatment and the lack of knowledge of the mechanism, no one treatment can be recommended with enthusiasm. Procedures which have been reported as effective in some cases and failing in others are the intravenous administration of alkalis, hypertonic dextrose solutions intravenously, roentgen ray irradiation or diathermy to the kidney regions, lavage of the renal pelvis with hot water, spinal anesthesia, transfusions of compatible blood, and decapsulation of the kidneys.

Prognosis

Hemolytic reactions should be seriously regarded, but the prognosis is extremely variable, depending probably on a number of factors. The amount of hemolyzed blood is undoubtedly important as well as the dose per minute.

PRESERVED BLOOD

The preservation of whole blood for transfusion has made possible the separation of the donor from the recipient in space and the remote dissociation of the act of blood collection from that of administration in point of time. These are desirable features in the mechanics of blood transfusion because they make possible the maintenance of stores of blood known as "blood banks." The chief advantages of the operation of a blood bank are: (1) A quantity of whole blood, previously grouped and Wassermann-tested, is immediately available for transfusion in emergencies. (2) The relatives or friends of a recipient are enabled to exchange their blood, of whatever group, for the blood of the proper group received by the patient. (3) Plasma is a by-product of preserved blood in the bank and may be employed for suitable indications.

Operation of a Blood Bank

A blood bank is best operated in conjunction with a hospital or a series of related hospitals. A "capital" of from 25 to 50 flasks of blood with suitable proportions of all blood groups should be acquired and maintained at all times in a refrigerator where the temperature is controlled at 2 to 5 C. As withdrawals for transfusions are made from the bank, equal quantities of blood of any group should be obtained from relatives or friends of patients in replacement. Transfusions of homologous groups should be made to maintain the proper proportion of blood groups in the bank. A number of financial and replacement arrangements are possible.

An important detail in operation is the method of assigning responsibility for the procurement of donors. Three systems are possible, each with its advantages and its disadvantages. Each service in the hospital or each hospital in a series may have its "account" with debits and credits in quantities of blood. This works well where the services are well organized and supervised. The surplus accumulated by a service may be sufficiently large to allow diversion of occasional blood transfusions to the patient who can procure no donors. Another method is to maintain an account for each intern or house officer. This has the advantage of placing responsibility directly on the physician for the procurement of donors. Its disadvantage is that the service suffers for the presence of an uncooperative physician. The individual surpluses are never large and can be used with difficulty for the recipient who can procure no donors. The third arrangement is to charge each patient receiving transfusions with the blood received and allow him to make

icterus is not definitely established as noninfectious. Laboratory studies have demonstrated that *Treponema pallidum* in citrated blood is not viable after storage in the refrigerator for seventy-two hours. Much longer periods of storage, however, will not apparently kill malarial parasites.

Serum Sickness and Drug Sensitivity

Etiology

The cause of serum sickness occurring after transfusions of human blood is not known although it has rarely been reported. Drug sensitivity has been passively transferred in animals by transfusion, and it seems logical that it could also occur in human beings.

Clinical Description

I have observed two cases in which the typical manifestations of serum sickness developed, in one patient four days, in another seven days, after blood transfusion. The disease was marked by fever, urticaria, arthrosis, and painful lymphadenopathy. The course in each was typical. Neither donors nor recipients had ever received horse serum.

A third case illustrates what is apparently another mechanism. A donor had been under treatment for a streptococcic pharyngitis and had received sulfathiazole, which had produced an erysipeloid eruption necessitating discontinuance of the drug. One month later, blood from this donor was transfused into a moribund patient with a severe genito-urinary infection. The patient had been receiving sulfathiazole without manifesting any toxic reaction. While the transfusion was being administered, the recipient suddenly developed a generalized erythema and massive edema covering most of the body. The patient died, probably from the infection, before the course of the transfusion reaction could be determined. This case is interpreted as being an example of passive transfer of drug sensitivity. With the widespread use of sulfonamide therapy it may be anticipated that such a combination of circumstances will become more frequent.

Diagnosis should not be difficult if the possibility is borne in mind.

Treatment is symptomatic.

Prognosis cannot be stated from the paucity of experience so far.

Prophylaxis is uncertain, but it seems logical to prevent persons who have acquired sensitivity to drugs from giving blood to recipients receiving the same drugs.

the emergency treatment of shock from hemorrhage. Should the plasma be considered of prime importance, technics should be adopted which alter the plasma less but at the same time preserve red cells for a much shorter period. The red cells then become an incidental asset during a short period of storage, during which the whole blood may be used if occasion demands. The plasma is then processed and the red cells are either used within a short time in resuspended form or are discarded. In this type of arrangement the erythrocytes are actually the by-product of the system. It should be clearly understood that the latter system deliberately substitutes a large supply of plasma for the emergency treatment of hemorrhagic shock when the discarded whole blood would be more efficacious. The arrangement may be justified, however, on the grounds of inadequate facilities for grouping and crossmatching blood or for purposes of long storage.

Changes in Blood During Storage

In most preservative mixtures the red cells remain intact longer when the temperature is maintained below 10° C. The usual temperature of storage is accepted as 2 to 5° C. Hemolysis proceeds relatively rapidly in citrated blood. The rate of disintegration is accelerated by the addition of sodium chloride. Hemolysis is retarded by the addition of dextrose, and it is now accepted that some combination of citrate-dextrose is desirable for preservation of whole blood. The available evidence indicates that the addition of dextrose solutions to citrated blood impedes hemolysis by a dual effect. The sugar, even in concentrations as small as 300 mg. per 100 cc., furnishes a substrate for glycolysis and keeps the formation of inorganic phosphorus at a minimum. A further inhibitory effect is obtained by the dilution of the plasma by a nonelectrolyte such as a solution of dextrose. The greater bulk not only permits longer storage but lessens the precipitation of fibrin in the plasma so that filters in administration apparatus employing a "closed system" are not so readily plugged. Loutit, Mollison, and Young²⁷ demonstrated that dextrose-citrate solutions apparently preserved blood better *in vitro* and *in vivo* when the pH of the solution was adjusted between 7.1 and 7.3 by the addition of citric acid. They studied a series of such mixtures and found several which were satisfactory. Various solutions of trisodium citrate, dextrose, and citric acid are now widely employed in the United States under the name ACD (acid-citrate-dextrose) but there is great variation in the formulae. The pertinent facts on several blood mixtures are summarized in the accompanying table.

restitution in kind or in money. This system is most practical where no indigent patients are treated.

Centralization of the laboratory tests and the custody of the blood is desirable for smooth operation of a blood bank. Collection of the blood can well be performed under the direct auspices of the transfusion service. Administration of the blood may be left to the individual physicians who have immediate care of the patients. It is most satisfactory to entrust the grouping and crossmatching of blood only to experienced technicians. Although the tests may appear simple in principle, they actually require considerable care and knowledge for accurate results.

It has been the experience in many institutions that the number of blood transfusions has more than doubled when a bank has been established. This should be recognized when plans are made for the physical plant. Complete success in operation of a blood bank cannot be attained unless the director of the transfusion service possesses and exercises full authority to refuse demands for blood when an appropriate balance does not exist in the bank for the service making the request. Many urgent situations arise, and although the physician is laudably concerned that his patient should receive prompt and efficient treatment, he may be inclined to regard the securing of donors to cover the transfusion as an unnecessary detail. Such an attitude, if allowed to persist, will obviously result in failure of the blood bank principle.

Technic of Preservation of Blood

It is not within the scope of this article to present all of the details involved in the storage of blood for transfusion but rather to consider some of the principles. More detailed treatment will be found in a technical manual of the Office of Civilian Defense entitled *The Operation of a Hospital Transfusion Service* (OCD Publication 2220).

There are now available many mixtures in which blood may be stored at 2 to 5° C. for transfusion. The selection of one for use in a particular situation should be governed by the following considerations

Primary and Secondary Purposes of the Blood Bank

If it is desired to furnish whole blood transfusions for the treatment of anemia and to provide maximum therapy for exsanguinated patients, methods should be adopted which will best preserve erythrocytes for the longest period of time. The plasma from the out-dated blood then becomes a by-product which is efficacious in the correction of hypoproteinemia and in

Some slight inhibition of hemolysis may also be obtained by displacing all of the air in the collecting flask with the blood mixture. The agitation of flasks of blood incurred in usual handling or in transportation results in no demonstrable damage to the red cells.

During storage the potassium of the erythrocytes diffuses into the plasma and is replaced by the sodium from the plasma. The organic phosphorus of the cells is changed to the inorganic form, which slowly diffuses into the plasma. This process is much reduced when added dextrose is present. The erythrocytes gradually swell during storage, and their resistance to hypotonic saline solutions becomes less. In solutions to which considerable quantities of dextrose are added, some of this change may be ascribed to diffusion of dextrose into the cells. The dextrose may be washed out and the osmotic fragility partially restored to normal.

The changes in the plasma during the preservation of whole blood are less important. The albumin and globulins change very little in the short time. Prothrombin is inactivated slowly, being about 70 per cent of normal in twenty-one days and about 30 per cent of normal in thirty days. Complement survives about fifteen days. Antitoxins are apparently little affected.

The leukocytes are quickly autolyzed, probably most of them are disintegrated in two days. The platelets are even more ephemeral, lasting only a few hours. The fate of the thromboplastin released from the disintegrated platelets is unknown.

BLOOD DERIVATIVES

Erythrocyte Suspensions

The most convenient source of suspensions of red cells for transfusion is a program for the processing of plasma. The whole blood is collected, citrated, and centrifuged and most of the plasma aspirated from the flask leaving a layer of packed erythrocytes. These may be resuspended in isotonic saline, grouped, tested for sterility and for hemolysis, and employed in transfusions. It is preferable to keep the cells unsuspended at 2 to 5° C. until ready for use before adding the saline. It is not desirable to employ cells so stored more than five days after collection. Red cell transfusions are extremely useful in the treatment of anemia. Because of the lack of plasma doses as large as 1 or 2 liters may be given in one transfusion without embarrassing the circulation. This provides a more rapid method of increasing the hemoglobin of the anemic patient than by the transfusion of whole blood.

CONSTITUENTS AND CRITIQUE OF SEVERAL BLOOD PRESERVATIVE MIXTURES

	Blood-Citrate	Denstedt's	Alsever-Amslie	Modified Rous-Turner (DeGowin et al)	Acid-Citrate-Dextrose (ACD)
Blood					
Trisodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 + 2\text{H}_2\text{O}$)	500 cc. 70 cc (2.5%) or 50 cc (4%)	500 cc 100 cc (3.2%)	500 cc 80 Gm.	500 cc. 100 cc. (3.2%)	500 cc 1.66 Gm.
Citric Acid ($\text{C}_6\text{H}_5\text{OH}(\text{COOH})_3 + \text{H}_2\text{O}$)	none	none	none	0.46 Gm.	0.6 Gm.
Dextrose (anhydrous)	none	150 cc. (5.4%)	18.66 Gm	700 cc. (5.4%)	3.7 Gm.
Sodium Chloride	none	none	4.18 Gm	none	none
Water	—	—	500 cc.	—	125 cc.
Blood/diluent ratio	0.14 or 0.1	0.5	1.0	1.4	0.25
Average Outdating Period (days)	5 to 10	18	18 to 21	21 to 30	21 to 30
Advantages	Compactness Minimum material added to plasma	Compactness Added storage time	Added storage time Plasma by sedimentation Minimum fibrin precipitation	Added storage time Plasma by sedimentation Minimum fibrin precipitation	Added storage time Compactness
Disadvantages	Minimum storage time Maximum precipitation of fibrin Poor plasma yield by sedimentation	Much precipitation of fibrin	Bulky for children	Bulky for children	Much fibrin precipitation Poor plasma yield by sedimentation

NOTE: Solutions of dextrose become caramelized when heated with an alkali like trisodium citrate. When autoclaved together the pH should be buffered with phosphates, as in the solution of Muehler and Andrews, or the alkalinity should be adjusted by the addition of citric acid

Blood Serum

The serum from coagulated human blood is interchangeable therapeutically with plasma except when prothrombin is desired. The processing is somewhat easier than for plasma, and serum may be forced through bacterial filters where plasma may not. The disadvantage is that the red cells cannot be employed for transfusions as a by-product of the method.

Human Serum Albumin

Human plasma or serum may be subjected to a physicochemical fractionation process developed by Cohn²³ which produces a solution of plasma proteins that is more than 99 per cent albumin. Serum albumin is soluble in nearly any concentration and is extremely stable in the liquid state, forming no precipitate when stored at tropical temperatures over long periods of time. The albumin of the plasma exerts about 80 per cent of the total osmotic pressure of the proteins. It may therefore be employed in the treatment of secondary shock instead of plasma. It was packaged for the armed forces in a 25% solution. This is four times isotonicity, this concentration was employed because of the compactness of the package. One hundred cubic centimeters of solution will exert approximately the same osmotic effect as 500 cc. of plasma. Its advantage is its compactness for certain military purposes. The disadvantages are the expense of processing and the hypertonicity. The clinical trial of a salt-poor human serum albumin solution is now in progress in the treatment of nephrotic edema and certain other conditions.

Human Plasma Globulins

With the fractionation methods developed by Cohn the antibodies against measles which are present in pooled plasma in great dilution can be concentrated to such an extent that a small dose is equally effective, if not superior, to the placental extracts as a prophylaxis against measles. Another fraction of the globulins can be concentrated by chemical methods so that the titer of the isohemagglutinins is greatly increased. This product can be employed for the manufacture of blood grouping globulins on a large scale. A material can be isolated from the globulin fraction which decreases the coagulation time in hemophiliacs when the substance is given intravenously.

Human Fibrin

Human fibrin can be isolated from the plasma by chemicophysical methods described by Cohn and can be fashioned into strong films which have proved

Blood Plasma

The transfusion of citrated human blood plasma is indicated for the treatment of hypoproteinemia and in the treatment of shock from burns. It is a substitute for transfusions of whole blood in the emergency treatment of shock from hemorrhage but is distinctly inferior to the latter. In severe grades of exsanguination there is a greater tendency for relapse to occur when the patient is treated with plasma than when he receives whole blood.

Plasma may be obtained by sedimentation or by centrifugation of preserved whole blood. It is preferable to centrifuge citrated blood, but for sedimentation, the blood mixtures which cause appreciable dilution of the plasma are better because the yield of the aspirated plasma protein is greater. Sedimentation is the ideal method where plasma is regarded as a useful by-product of a blood bank. When the whole blood is out-dated, the plasma may still be separated and stored.

Liquid plasma may be stored in the refrigerator at 2 to 5° C. for short periods of time but is better stored at room temperature, when the precipitation of fibrin is slower than at lower temperatures. It may be stored at room temperature for two years, and is effective in the treatment of shock after that length of time.

Plasma may be kept in the frozen state indefinitely, preferably at temperatures of minus 15 to 20° C. Just before use it should be thawed in a water bath at 37° C. When it is allowed to stand for a few hours after thawing, a heavy precipitate is formed. The advantages of frozen plasma are that precipitation of protein and bacterial growth are inhibited, complement and prothrombin are preserved. The disadvantages are the added expense of storage in the frozen state and the time consumed in thawing during an emergency.

Dried plasma will keep indefinitely in a sealed container at room or tropical temperatures. The plasma is dried from the frozen state by withdrawing the moisture by creation of a high vacuum. When properly dried, with a moisture content of less than 1 per cent, it may be completely dissolved in thirty seconds when sterile distilled water is added. Various concentrations of plasma may be reconstituted, up to four times isotonicity, by adding less water than was withdrawn. The disadvantages of dried plasma are the added expense of processing and the large bulk of storage space required. The flask in which the dried powder is stored must contain sufficient space to accommodate the reconstituted material, and a flask of sterile distilled water accompanies the plasma for reconstitution.

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superior to other substances as a substitute for dura mater in neurosurgery. The fibrin has also been produced in the form of a sponge or film which, when impregnated with thrombin, is employed in neurosurgery as a hemostatic agent.

Human Thrombin

Thrombin may be isolated from the plasma by Cohn's methods and may be employed as a hemostatic agent in neurosurgery and also, in combination with fibrinogen, as a cementing agent in skin grafts.

Human Globin

Strumia has devised methods for the production of solutions of globin from human erythrocytes which have colloidal properties and are proposed for intravenous injection to restore the blood volume in secondary shock. This application must still be regarded as experimental.

Human Hemoglobin Solutions

Amberson has proposed the transfusion of solutions of human hemoglobin to increase the oxygen capacity after hemorrhage. This must be considered as dangerous until more is known about the toxicity of hemoglobin.

Blood Derivatives from Heterologous Species

Historically the transfusion of blood from other species into man was occasionally practiced for hundreds of years but was finally abandoned because of the dangerous and fatal reactions frequently ensuing. Recently attempts to employ transfusions of bovine plasma or bovine albumin to restore the blood volume in shock have been made. They must at present be regarded as experimental. Bovine thrombin may be employed as a hemostatic agent and as a cementing substance in skin grafts.

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using fresh blood as it is difficult to resuspend the erythrocytes evenly after they have once settled. Erythrocyte counts and hemoglobin determinations are apt to be slightly low when oxalated blood is used.

BLOOD COUNTING

The hemacytometer (Fig. 85) consists of two pipets, one for the erythrocyte and one for the leukocyte count, a counting chamber with two ruled areas separated by a moat, and cover slips which are ground perfectly flat and are of greater thickness than those in routine laboratory use. The lines

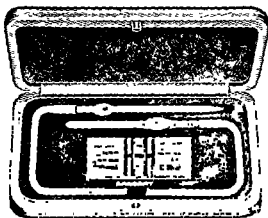


Fig. 85. Hemacytometer, consisting of counting chamber with double ruling and two pipets for diluting blood (E. H. Sargent and Co.)

on the floor of the counting chamber may have been made directly on the glass or upon a thin metallic coating which covers the floor of the chamber. This latter "bright line" instrument (Fig. 86) makes the ruling more easily visible, the lines appearing as bright streaks upon a darker background.

The pipets are not made to hold an exact amount of fluid or blood but the ratio between blood and diluting fluid is very accurate. Since the diluting fluid which remains in the stem of the pipet does not become mixed with the blood, the pipets are marked for 101 and 11 parts rather than 100 and 10. When blood is drawn to the 0.5 mark and diluting fluid to the 101 mark in the red cell pipet, it gives a dilution of 1 to 200. In the white cell pipet blood drawn to the 0.5 mark and diluting fluid to the 11 mark gives a dilution of 1 to 20.

The counting chamber is so constructed that the depth of the layer of

HEMATOLOGIC METHODS

OBTAINING BLOOD

THE SMALL AMOUNT OF BLOOD REQUIRED FOR MAKING A SMEAR AND BLOOD count is obtained from a puncture of the ball of a finger or lobe of an ear in adults or of the heel or toe of an infant. The procedure is less painful if the lobe of the ear is used, but a finger is more accessible and easier to manipulate.

The area selected for puncture should be cleansed with alcohol and wiped dry with a piece of gauze or cotton. The puncture should be made with a lancet or other sharp instrument which will make a short linear incision 2 to 3 mm. in length and about 2 mm. deep. The instrument must be sterile. An automatic lancet is a satisfactory instrument which can be set to make an incision of a given depth and is sprung by a trigger mechanism. A narrow, sharp-pointed knife blade (Bard-Parker No. 11) is also satisfactory. This may be kept in the cork of an alcohol bottle with its point projecting far enough through the under surface to be ready for instant use, the cork serving as a handle and guard. A venipuncture needle is not satisfactory since the wound it produces is small and round and closes quickly. The incision should be large enough so that a drop of blood wells up immediately but not large enough so that blood drips from the finger. No more than very slight pressure should be used to express blood as further manipulation results in admixture of tissue juice with the blood. If the incision is made on the ear, it should be on the edge rather than on the flat lateral surface. An incision on the ball of the finger should be a little to one side rather than in the middle and not at the tip. The first drop of blood should be wiped away with a dry gauze, and each specimen used for a count or smear should be from a fresh drop, the remaining unused portion being wiped away each time. The skin surface must be dry so that the drop of blood will well up and not spread out over a moist surface. If desired, blood counts and smears may be made from oxalated blood obtained by venipuncture, but this is less satisfactory than

From a puncture wound on the finger or ear draw blood exactly to the mark 0.5 on the stem of the red cell pipet, wipe the tip of the pipet clean with a nonabsorbent material, and fill to the mark 101 with diluting fluid. After shaking the pipet for a few seconds, lay it aside (preferably on a level rack so the fluid does not run out) until blood is obtained for the leukocyte count and smear. Before filling the counting chamber shake the pipet for a full three minutes, holding the ends between the thumb and finger, so that the cells and diluting fluid are thoroughly and evenly mixed. Discard the first two or three drops before filling the counting chamber.

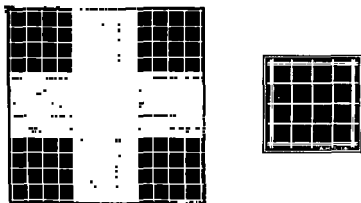


FIG. 87. Improved Neubauer's Counting Chamber. (Courtesy, F. A. J. Co., Cambridge, Mass.)

The erythrocyte count is made in the central part of the ruled area of the counting chamber using the high dry objective of the microscope. The smallest squares, which are used in the red cell count, are $1/20$ mm. square and are arranged in groups of 16 (Fig. 87). Each group is surrounded by a heavy line, double lines, or triple lines depending upon the type of ruling on the chamber. Count the erythrocytes in 5 blocks, each of which contains 16 of the smallest squares. It is best to count the 4 corner blocks and one of the central blocks so that a total of 80 of the smallest squares are counted. Record the number of erythrocytes in each block of 16 squares, which, in normal blood, will contain from 90 to 100 cells. The variation in the number of cells per block of 16 small squares should not be over 10. To avoid confusion it is best to count the cells lying on the upper and left boundary line of each square as being within the square and disregard those lying on the lower and

blood between the floor of the chamber and the under surface of the cover slip is exactly 0.1 mm.

To fill the counting chamber preparatory to making a count, place the instrument on a firm level surface and lay the cover slip in place. Both the floor of the counting chamber and the cover slip must be perfectly clean and free of grease. Blow out and discard two or three drops of diluted blood from the pipet and then expel enough of the suspension so that a very small drop hangs to the tip of the pipet.



FIG. 86 Counting chamber with "bright line" ruling. (E. H. Sargent and Co.)

Touch this drop of fluid to the aperture between the cover slip and the floor of the counting chamber. The fluid is drawn into the chamber by capillary attraction and will fill the chamber evenly and without air bubbles if the surfaces are clean. Too large a drop of diluted blood results in an overflow into the surrounding moat. This should be avoided. Allow the fluid to settle for a few minutes so that all the cells will be on the floor of the chamber and in the same focal plane when examined microscopically.

Erythrocyte Count

The two most commonly used diluting fluids for erythrocyte counts are Toison's and Hayem's. Either is satisfactory. Both solutions must be filtered occasionally to rid them of fungi.

Hayem's solution

Mercuric chloride	0.5
Sodium sulfate	5.0
Sodium chloride	1.0
Distilled water	200.0

Toison's solution:

Sodium chloride	1.0
Sodium sulfate	8.0
Glycerin	30.0
Distilled water	160.0

Add enough methyl violet to give a light purple color.

Accurate counting of blood cells requires a technic which can be acquired only with practice. The ability to stop the diluting fluid exactly at the desired mark on the pipet cannot be attained by reading directions in a textbook.

In a well mixed suspension the variation in the number of cells per square should not be over 10. To obtain the number of cells per cubic millimeter of blood multiply the total number of cells in the 4 squares by 50 (Divide the sum by 2 and add 2 ciphers.) Since each corner area is 1 mm. square, the depth of the fluid 0.1 mm., and the dilution in the pipet 1:20, the factor is obtained as follows:

$$1 \times 1 \times 1/10 \times 1/20 \times 4 = 1/50$$

Cleaning the Instruments

Wash the counting chamber and cover slip with water or alcohol and dry with a soft cotton cloth or gauze.

Pipets should be cleansed by drawing successively distilled water, alcohol, and ether or acetone through them. Air should be drawn through the pipet until free movement of the glass bead indicates that it is dry. This is best done by means of a suction apparatus. Do not blow through the pipet with your mouth as moisture will condense in the bulb.

Coagulated blood may be removed from the bulb of the pipet by allowing hydrogen peroxide to stand in it for a short time. If this is not successful, strong nitric acid may be used, but do not allow this to come in contact with the paint in the markings on the stem of the pipet. Foreign material in the stem of the pipet may be dislodged with a horse hair or fine wire.

HEMOGLOBIN DETERMINATION

The hemoglobin content of the blood should always be reported as grams of hemoglobin per hundred cubic centimeters of blood. It should never be expressed as percentage of normal unless the statement includes the normal value, in grams, from which the percentage was computed.

Oxygen Capacity Method

The Van Slyke modification of the Haldane method for determining the oxygen-absorbing capacity of the blood is used to standardize other methods and instruments used in hemoglobin determinations. It is not applicable for routine clinical use. This method measures (by means of an apparatus for gas analysis) the amount of oxygen required to saturate a known amount of blood. One volume per cent of oxygen is equivalent to 0.746 Gm. of hemoglobin, or 1.34 cc. of oxygen combines with 1 Gm. of hemoglobin.

right boundary lines. Each small square is $1/20$ mm. on each side, the depth of the chamber is 0.1 mm, the dilution is 1:200, and 80 squares are counted so that the number of cells per cubic millimeter of blood is calculated from the formula:

$$1/20 \times 1/20 \times 1/10 \times 1/200 \times 80 = 1/10,000$$

Multiply the number of cells found in the 80 squares by 10,000 or simply add 4 ciphers to the number of cells counted. If a different dilution is employed in the pipet, as 1:100, the formula is changed accordingly.

Leukocyte Count

The diluting fluid used for the leukocyte count is acetic acid, from 1 to 5 per cent. A small amount of methylene blue may be added so that the solution is slightly colored. The dye not only serves to distinguish the fluid but also makes the nuclei of the cells stand out more plainly in the counting chamber.

Draw blood into the "white" pipet to the mark 0.5 and diluting fluid to the mark 11. A much larger drop of blood is necessary to fill the leukocyte pipet than the red cell pipet. The color of the fluid changes to brown almost immediately after mixing because acid hematin is formed after hemolysis of the erythrocytes by the acetic acid. Shake the pipet for three minutes, discard the first two or three drops, and fill the counting chamber just as in doing an erythrocyte count. Because of the larger bore in the stem of this pipet the fluid runs out more freely than with the red cell pipet, and the danger of flooding the stage of the counting chamber is greater. If the opposite side of the stage of the counting chamber is being used for an erythrocyte count, be sure that the two fluids do not come in contact with each other. Allow the slide to stand until the cells have settled to the floor of the counting chamber. The count is made with the low power (16 mm.) objective. Under the microscope the leukocytes appear as small black dots (nuclei), which are either round or irregular in shape, with a bright halo (cytoplasm) around them. Use the high power objective of the microscope when it is necessary to identify a cell as specks of dirt may cause some confusion.

The entire ruled area on a counting chamber is 9 sq. mm. In each of the 4 corners of the ruled area is a large square subdivided into 16 smaller squares (see Fig. 87). The leukocytes are counted in the 4 large corner squares which are 1 mm. square, each of which nearly fills the field of vision with the low power objective. Record the number of cells in each of the 4 large squares

blood or the percentage of hemoglobin, the scale on one side of the tube reading in grams and on the other side the percentage of normal. In the Sahli instrument 13.8 Gm. is considered to be normal while in the Sahli-Hellige instrument 14.5 Gm. is used as the normal.

Haden-Hausser Hemoglobinometer

The Haden-Hausser Hemoglobinometer consists of a movable carrier with individual glass standards representing from 7.5 to 18 Gm. of hemoglobin. Beside the glass standard is a channel of varying depth for the unknown acid hematin solution. The reading is made through an attached magnifying lens (Fig. 90). Blood is diluted 1:20 and thoroughly mixed with tenth-normal hydrochloric acid in a leukocyte-counting pipet and allowed to stand for thirty minutes. The solution is placed in the channel of the movable carrier and the color matched with the glass standards. The result is read in grams of hemoglobin per hundred cubic centimeters of blood from that standard with which the color of the solution matches. If the hemoglobin is below 7.5 Gm., it is necessary to make a 1:10 dilution of the blood and correct the reading from the standard accordingly. One advantage of the Haden-Hausser instrument is that a leukocyte count can be made on the same specimen of diluted blood from which the hemoglobin is determined.

Newcomer Method

The Newcomer method employs a standard brown glass disk which is placed in one of the light paths of a Duboscq type of colorimeter. The cup on the side in which the disk is placed is filled with diluted water. Twenty cubic millimeters of blood is diluted to 5 cc. by tenth-normal hydrochloric acid in a special pipet, and this acid hematin solution is placed in the opposite cup of the colorimeter. The colors are matched by raising or lowering the cup containing the acid hematin and the micrometer scale is read as in any colorimetric examination. The manufacturers of the instrument supply a chart which indicates the grams of hemoglobin represented by each division of the micrometer scale. Each Newcomer disk has a correction factor, and

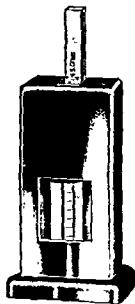


FIG. 89. Sahli type hemoglobinometer. A square tube for the unknown solution is graduated to read in grams of hemoglobin per hundred cubic centimeters of blood. A glass prism standard is placed on each side of the unknown (A. S. Aloe Co.)

Tallqvist Scale

The Tallqvist scale is probably the most widely used and the most inaccurate of all methods of hemoglobin determination (Fig. 88). A drop of blood is placed on a piece of absorbent paper and allowed to dry. The color is then compared with a color scale which represents varying percentages of hemoglobin concentration. This method is not acceptable.

Acid Hematin Methods

In all the modifications of the acid hematin method hemoglobin is converted to acid hematin by the addition of an acid. Acid hematin is brown, and the intensity of the color is compared to that of a known concentration of acid hematin.

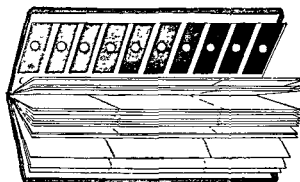


FIG 88 Tallqvist scale (A H Thomas Co)

Sabli Hemoglobinometer

The Sahli Hemoglobinometer consists of a case containing an upright brown glass standard with an adjacent graduated tube for the unknown solution of acid hematin (Fig. 89). The color of the unknown solution is compared to that of the standard while both are standing in front of an opaque glass background. Tenth-normal hydrochloric acid is placed in the graduated tube to the mark 10. A special pipet is used to collect 20 cu. mm. of blood which is thoroughly mixed with the acid in the graduated tube. The mixture is allowed to stand for 10 minutes for full development of the color. Distilled water is added drop by drop and thoroughly mixed with the solution until the color of the fluid exactly matches that of the standard. The upper level of the fluid is then read on the scale etched on the tube. This is graduated to read directly the grams of hemoglobin per hundred cubic centimeters of

the solution of acid hematin must stand at least thirty minutes before being read. A special Newcomer colorimeter is obtainable on which the micrometer scale of the instrument is calibrated to read directly in grams of hemoglobin per hundred cubic centimeters of blood (Fig. 91).

Photometer

There are several instruments on the market which utilize a photoelectric cell for the estimation of hemoglobin, using either an acid hematin or an oxyhemoglobin solution (Fig. 92). These are rather expensive but they are accurate, easy to operate, and require little time. With proper filters they may be used for other colorimetric determinations in addition to their use for hemoglobin determinations.

BLOOD SMEARS

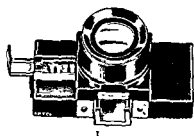
A microscopic examination of a blood smear should be made on all patients. The usual preparation for this examination is a fixed blood smear stained by one of the polychrome or modified Romanowsky stains which combine basic methylene blue and acid eosin stain. The most common stains of this type are Wright's and Giemsa's. Other types of stain are used for the study of certain specific features of blood cells. These may be used on fresh blood, as brilliant cresyl blue for reticulocytes or neutral red and Janus green for supravital staining, or on fixed smears as with peroxidase stain.

Making the Blood Smear

The glass slides used for making a blood smear must be thoroughly cleaned, preferably washed with soap and water, rinsed in 95% alcohol, and dried with a lint-free cloth. A dirty slide causes an uneven smear, and grease, as from a fingerprint, causes a moth-eaten appearance. Always pick up slides by their edges so as not to touch the surface with the fingers.

Touch the surface of a slide, near one end, to a drop of blood and place it on a flat firm surface. Place the end of a second slide across the surface of the first slide ahead of the drop of blood and at an angle of about 30 degrees. Draw this spreader slide back until it comes in contact with the blood, hold it stationary until the blood has spread across the width of the slide, and then push the spreader slide forward with a firm steady motion. In this way the blood is drawn along the slide rather than pushed.

As an alternate method put the slide on which the smear is to be made on a firm flat surface. Obtain a drop of blood on the end of the spreader slide,



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FIG 90. Haden-Hausser hemoglobinometer 1. Rear view showing light filter in place. 2. Comparator slide with cover glass in position. (A. H. Thomas Co.)

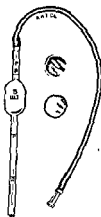
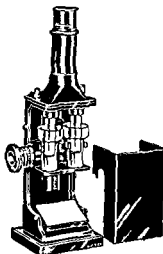


FIG 91. Newcomer hemoglobinometer, diluting pipet, and standard disk. The hemoglobin in grams is read directly on the micrometer scale. (A. H. Thomas Co.)

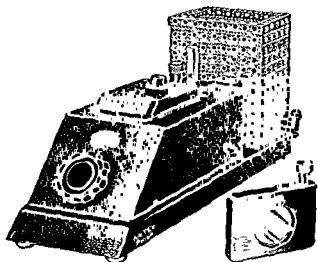


FIG 92. Photoelectric colorimeter for hemoglobin determinations. (A. H. Thomas Co.)

Place a small drop of blood on the surface of a cover glass (No. 1, 7/8 inch square) which has been carefully cleaned. Drop another cover glass on the drop of blood in such a position that the corners of the cover glasses do not coincide. Allow the blood to spread in a thin film between the two cover glasses, and as soon as the blood film reaches its maximum size, pull them apart, keeping their surfaces parallel. Allow the smears to dry in air without heating. Cover slip smears may be placed on small corks, which are standing on end, while staining and are mounted on slides for examination.

Staining the Blood Smear

Wright's Stain

The most satisfactory stain for routine use on blood smears is Wright's stain. It may be prepared from dry powder or purchased in a prepared form ready for use. It deteriorates rather rapidly and must be kept in small dark brown bottles which are tightly stoppered. It should be exposed to air as little as possible. With some Wright's stains it is necessary to use a buffer solution as a diluent in order to maintain a proper pH and obtain a satisfactory staining reaction. When a buffer solution is necessary, each new batch of stain may require some alteration in the pH of the buffer solution in order to obtain the desired results. Some Wright's stain may be diluted with fresh distilled water in place of a buffer solution. We are accustomed to use a commercially prepared stain (Coleman and Bell) without a buffer solution.

Preparation of Wright's Stain

0.1 Gm. Wright's dye

60 cc. pure acetone-free methyl alcohol

With a clean mortar and pestle grind the dye with alcohol, adding the alcohol very slowly until all of the dye is in solution. Place in a dark bottle, allow to stand at least twenty-four hours, and filter.

A buffer solution is prepared as follows:

Potassium phosphate (monobasic)	6.63 Gm
Diabasic sodium phosphate	2.56 Gm.
Distilled water	1000.00 cc.

Staining Technique. Place the blood smear on a level staining rack. This may consist simply of two rods placed across a sink or any type of open vessel. Do not stain a smear with the slide lying on a flat surface. It is essential • that the rack be level, otherwise the stain will gravitate to one side or one end of the slide and result in a concentrated stain in one area and a dilute stain in another, and portions of the smear will be overstained or under-

place that end on the surface of the first slide near one end so that the blood spreads across the slide and is contained in the angle away from the direction of motion. Spread the same as above with a quick firm motion (Fig. 93).

The smear should be allowed to dry in the air, and drying should be complete within a few seconds in a properly made smear. The slide may be labeled by writing with an ordinary pencil on the thick end of the blood smear. At least two good smears should be made on each patient.

A good smear is thin, and the cells are evenly distributed. It is nearly as wide as the slide on which it is made and covers from one-half to two-thirds the length of the slide. At the starting end, where the blood was first applied

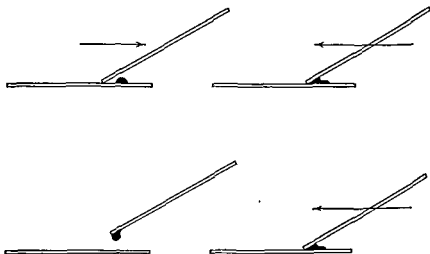


FIG. 93 Methods of spreading the blood in preparing a blood smear.

to the slide, the smear is thickest and is unsatisfactory for differential counts, but it gradually thins out toward the distal end. The drop of blood was too large when the smear extends the whole length of the slide, and the smear will be too thick. If the smear is thick, dark red in color, and without a thin even distribution of the cells at one end, it should be discarded. There is no use in staining a poorly made smear.

Cover Glass Method for Blood Films

Some workers believe that there is a more even and accurate distribution of cells and less distortion when blood smears are prepared on a cover slip. Such smears are more difficult to prepare and more troublesome to mount and, unless they are very carefully made, are less satisfactory than ordinary slides.

the myelocytic series of cells. The smear is first stained with Wright's stain by the usual method. The stain is washed off and the smear immediately immersed in diluted Giemsa stain where it remains for twenty minutes.

Examination of the Blood Smear

Examination of an improperly made or poorly stained blood smear should never be attempted. It is not only a waste of time but may be misleading. The smear should be thin, with the erythrocytes evenly distributed in a single layer and close together but not overlapping. The thin terminal portion of the blood smear is the most satisfactory area for study. Always examine the erythrocytes, noting their size, shape, staining reaction, and whether or not polychromasia or basophilic stippling is present. Note the relative frequency with which platelets are encountered.

Differential Count

A differential count must always be done with the oil immersion objective on a portion of the smear which is thin and has an even distribution of cells. A mechanical stage, while not necessary, greatly facilitates the examination and increases the accuracy of a differential count. Make a list of the various types of leukocytes and place a mark in the proper column as a cell of each particular type is encountered on the smear. Count all the cells which are seen, using an "unclassified" column for those you cannot identify, and under the heading "degenerated cells" include broken cells, basket cells, and naked nuclei. A total of 100 cells is usually recorded, and from this is determined the percentage of each of the constituent types. A differential count from 200 or 500 cells is more accurate.

There is a tendency for the larger cells, particularly the granulocytes, to be more abundant at the margins and at the end of the smear so that the method of examination should be designed to give a representative sampling of all portions of the smear (Fig. 94). After bringing the cells into focus, move the slide across the field of vision until the edge of the smear is reached, laterally through a few ocular fields along the outer margin of the smear, and then back across the smear to the other edge. Repeat this procedure until the required number of cells has been recorded. A second maneuver starts the examination along the edge of the smear, moves inward a short distance, parallels the margin, and again moves out to the edge of the smear. Repeat the procedure as many times as is necessary to identify the required number of cells.

stained accordingly. Apply enough Wright's stain with a dropper to just cover the surface of the slide. Allow this to stand for one minute. Dilute the stain by adding with a dropper as much distilled water or buffer solution as will remain on the slide without overflowing. About three times as much water as stain is required. A metallic sheen appears on the surface of the stain with this dilution. Allow the diluted stain to remain at least ten minutes. Remove the stain by flooding it off with distilled water from a wash bottle. Wipe off the under side of the slide with gauze and stand the slide on end to dry in the air. Gentle heat may be used to hasten drying, but do not get the slide hot. Do not blot the smear with any absorbent material to dry it.

Concentrated Wright's stain fixes the smear, but the staining properties before dilution are unimportant. Staining of the cells occurs only after the diluting fluid has been added. The best results and the clearest definition of cytoplasmic granules are obtained with a dilute stain which is allowed to stay on the smear for a long time (at least ten minutes). The distilled water should be neutral in reaction since excessive acidity accentuates the eosin of the stain at the expense of the basophilic properties, and an excessive alkalinity causes a staining reaction which is too dark and basophilic. In an understained smear the cytoplasm of the leukocytes may be stained but the nuclei will be unstained or very slightly stained. In an overstained smear the leukocytes are dark and too blue so that the cytoplasm and granules are not distinct.

Giemsa Stain

Another modification of the Romanowsky stain is the Giemsa stain. It is the best method when a large number of smears are to be stained.

Giemsa stain—stock solution.

Azure II eosin	30 Gm.
Azure II	08 Gm
Glycerin C. P.	2500 cc
Methyl alcohol	2500 cc

For staining, dilute 1 part of the stock solution with 10 parts of distilled water.

Fix the blood film by immersion in methyl alcohol for two or three minutes and then immerse in diluted Giemsa stain for twenty minutes.

Combined Wright's and Giemsa Stain

The combination of Wright's and Giemsa stain is sometimes used to bring out basophilic changes in the erythrocytes and to accentuate the granules in

RETICULOCYTE COUNT

There are several methods for staining reticulocytes, all of which require the living erythrocyte to be brought into contact with the dye. They cannot be stained on a previously fixed smear.

Staining Reticulocytes

Capillary Pipet Method

1. Prepare a supply of fine capillary pipets by heating glass tubing in a flame until soft. Pull the heated portion out into a long capillary tube. A satisfactory pipet has at least 3 cm. of the original tubing with a capillary tip several centimeters long. Draw staining fluid into the pipet, blow out the excess, and allow that adhering to the walls of the tube to dry. As many pipets as desired can be prepared at one time and kept for future use. The staining fluid consists of 5 cc. of a saturated aqueous solution of brilliant cresyl blue mixed with 1 cc. of 2% sodium oxalate, the latter being added to prevent coagulation of the blood in the tube. Draw a small amount of blood into the tube from a puncture wound and agitate back and forth in the tube a few times by alternate suction and blowing. Allow it to stand in the tube for twenty minutes. Spread on a slide as for an ordinary blood smear and allow it to dry. This may be counterstained with Wright's stain if desired. The reticulocyte count is somewhat lower when a dried stain is used, but this is a time-saving method when a supply of tubes is available.

2. A second method utilizing a capillary pipet consists in drawing a drop of blood into a clean pipet followed by a drop of the same dye used in method 1. Mix the blood and dye for a few seconds and allow the mixture to stand for twenty minutes. Make a smear as in the preceding method.

Moist Preparation

There are many methods whereby a drop of blood is placed directly in contact with a solution of the dye. The cells may then be examined in the moist state or they may be spread on a slide and allowed to dry. One very satisfactory method requires two solutions:

1. 0.3% solution of brilliant cresyl blue in 0.85% saline
2. 0.3% solution of sodium citrate in 0.60% saline

Mix one part of no. 1 with three parts of no. 2 just before use. To 1 cc. of this mixture in a centrifuge tube add a drop of blood. Shake for a few seconds and then allow the cells to settle to the bottom or centrifuge for twenty

As has been previously pointed out, the nucleus of the mature neutrophil has two or more lobules connected by narrow filaments of chromatin material. A few nonfilamented or band forms appear in normal blood, and it should be a routine practice to separate the neutrophils into band and segmented forms in all differential counts (see Fig. 10, page 63).

Schilling Count. The neutrophils are separated into myelocytes, juveniles (metamyelocytes), band (nonsegmented), and segmented forms, and each group is recorded separately in the differential count.

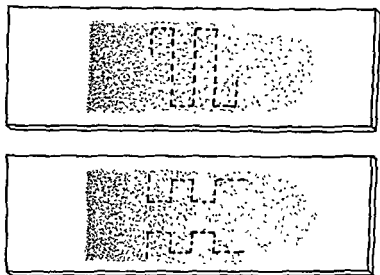


FIG 94. Methods of examining the blood smear in making a differential count.

Arneth Count. The neutrophils are classified according to their degree of nuclear segmentation. The young forms are classified as in the Schilling count but the segmented forms are further subdivided according to whether they have two, three, four, or five or more lobules. The percentage of each type is determined by counting 100 neutrophils. When the percentage of highly segmented forms is increased, a "shift to the right" has taken place. An increase in the percentage of immature, nonsegmented forms is termed a "shift to the left."

Filament-Nonfilament Count or Segment-Nonsegment Count. This consists in separating the band (nonfilament or nonsegment) cells and the filamented or segmented forms of neutrophils. It is a simpler classification than either the Schilling or the Arneth method.

Stains

Stock solutions of stain

Saturated solution of vital neutral red in absolute alcohol.

Saturated solution of Janus green (diazine green of National Aniline Company) in absolute alcohol.

Keep tightly stoppered in the dark.

Dilute stain for use on blood.

Dilute solutions do not keep well and should be freshly prepared as needed. Add 20 to 30 drops of the concentrated stock solution to 10 cc. of absolute alcohol. For leukemic blood it may be necessary to use from 40 to 70 drops to 10 cc. of alcohol.

Combination of neutral red and Janus green.

Add 3 drops of concentrated Janus green solution to 1 cc. of dilute neutral red solution.

Staining

Glass slides are scrupulously cleaned and dried. They are flooded with a dilute solution of dye, the excess is poured off, and that adhering to the slide is allowed to dry while the slide is in an upright position. Slides may be prepared for future use if stored in a dustproof box. A small drop of blood on a cover slip is inverted and placed on a warm slide which has been prepared with the stain. This is sealed with vaseline. The slide is placed on the warm stage and examined with oil immersion lens after ten minutes. The cells remain alive and actively motile for one to four hours.

Neutrophilic leukocytes are actively motile and contain numerous small red granules which constantly stream through the cytoplasm. Red digestive vacuoles are present, and the nucleus is usually in the rear of the moving cell. The granules of a dying cell do not take up the stain. Both eosinophils and basophils show the red granules.

Monocytes are rounded and are almost immobile. Fine red granules are grouped about a centrosphere, and many red digestive vacuoles will be found.

Lymphocytes show a clear cytoplasm with a few red vacuoles. With Janus green a clump of blue mitochondria is found opposite the indentation in the nucleus. There is usually no motility.

minutes Settling will require about two hours. A smear or a moist preparation is made from the sediment.

Dried Stain Method

Use 1% brilliant cresyl blue in ethyl alcohol. Cover one end (about one third or one fourth) of a glass slide with stain, removing the excess, and allow it to dry. A supply of slides may be prepared in advance.

Place a drop of blood on the stained area. Cover the blood and the stained area of the slide with a second unstained slide, the slides overlapping for about one third of their lengths. Allow this preparation to stand about ten minutes. At the end of this time smear the blood over the previously unused portion of the two slides and count the reticulocytes.

Oxalated Blood (Osgood-Wilhelm Method)

Mix equal parts of oxalated (or capillary) blood and 1% brilliant cresyl blue in 0.85% saline. Allow the mixture to stand for at least one minute. Make smears and allow them to dry in the air. If desired, counterstain with Wright's stain.

Counting Reticulocytes

In the eyepiece of a microscope place a paper or cardboard disk from the center of which a small square has been removed. This cuts down the field of vision and facilitates counting. Count the number of erythrocytes and the number of reticulocytes in the field of vision; move the slide to a new field and repeat until 500 or 1000 erythrocytes have been counted. Report the results as the percentage of erythrocytes which are reticulated.

The reticulocyte count ranges from 0.5 to 3 per cent in normal individuals with an average count of 1 to 1.5 per cent. The percentage is increased when erythrocytes are being regenerated rapidly and is lowered with decreased erythropoiesis.

SUPRAVITAL STAINING

For the study of living cells a moist preparation of blood is examined on a warm stage microscope. Certain dyes are used which stain the leukocytes but do not damage them or interfere with their motility. The various types of leukocytes may be recognized by the amount and the kind of dye that they take up, by its distribution within the cytoplasm, and by the degree and type of motility.

varying numbers of small dark dots which appear larger than those stained by Wright's method

Unna's alkaline methylene blue:

Methylene blue	1 Gm
Potassium carbonate	1 Gm.
Distilled water	200 cc.

Thick Drop Method

1. Place two drops of blood on a slide and spread to form a circle three times the diameter of the original drop. Dry for several hours in air or in an incubator. Cover the slide with diluted Giemsa stain and let stand for three minutes or until the hemoglobin begins to dissolve. Tilt the slide and allow the stain to drain off and again cover with dilute Giemsa stain. Allow this to stand for thirty minutes, flood the stain off with distilled water, and let the smear dry while standing on end. Examine with oil immersion lens. The erythrocytes are not fixed and consequently are hemolyzed by the dilute Giemsa stain. Only the inner structure of young erythrocytes and the stippling remain. The thick drop method is useful in examining blood for malarial parasites since each field in the thick drop preparation is equivalent to about fifty fields of the ordinary thin smear.

2. Spread a large drop of blood on a slide. Dry thoroughly in air or in the incubator for one-half to one hour. Put the slide in a solution of 5 per cent formalin in 1 per cent acetic acid for a few minutes. This takes out the hemoglobin and fixes the cells at the same time. Wash in water, dry, and stain with Wright's or Giemsa's stain. Counterstain for one minute in 2% methylene blue in 5% borax solution.

HEMATOCRIT

The hematocrit reading expresses the percentage of the total blood volume which is composed of packed erythrocytes. It is determined by centrifuging a specimen of blood to the point that further centrifugation does not diminish the volume of packed red cells. The length of time required for centrifugation must be determined for each centrifuge at certain speeds, usually 3500 r.p.m. Different types of tubes may be used including the ordinary conical centrifuge tube with volume graduations etched on the side. Five cc. of blood is placed in the tube, or a smaller amount if a small centrifuge tube is used, with an isotonic anticoagulant such as 1.3% sodium oxalate. The volume of the packed erythrocytes is ascertained after centrifuging the blood for the required time.

PEROXIDASE OR OXIDASE STAIN

Prepare a blood smear in the usual way. Cover the smear with a measured amount of Goodpasture's stain and allow it to stand one minute. Add an equal amount of freshly prepared hydrogen peroxide solution and let stand for four minutes. Rinse with distilled water, dry in air, and examine with the oil immersion lens.

Goodpasture stain:

Alcohol	100.00 cc.
Sodium nitroprusside	0 05 Gm
Benzidine C. P.	0 05 Gm
Basic fuchsin	0 05 Gm.

Dissolve the nitroprusside in 1 or 2 cc. of water, mix with the alcohol, and then add the other ingredients.

Hydrogen peroxide solution

1:200 dilution of hydrogen peroxide in water.

The granules of the myelocytic series of cells show the peroxidase reaction, becoming large dark-staining granules. Myeloblasts, which do not have granules, do not give the peroxidase reaction. A few of the dark granules are found in monocytes but they are not as large or as numerous as in the granulocytes. Lymphocytes do not show the peroxidase reaction and are pink in color. Neither myeloblasts nor lymphoblasts contain the peroxidase ferment so that they cannot be differentiated by this means.

Combined Peroxidase and Wright's Stain

Proceed with the Goodpasture stain as just described, rinse off the stain, but before drying stain with Wright's stain in the ordinary way.

BASOPHILIC STIPPLING

Basophilic stippling will be apparent with Wright's or Giemsa stain but is best demonstrated with methylene blue. Prepare a blood smear in the usual way but allow it to dry for twenty-four hours. Protect the smear from dust during the drying period. Cover the slide with Unna's alkaline methylene blue for two or three minutes. Remove the stain by flooding the slide very carefully with water. Since the smear is not fixed, except by the drying, the blood cells will be washed off easily. Erythrocytes stain a pale bluish color and only the periphery of the cell may be seen. Stippled corpuscles show

onto the field of vision. The scale readings must be standardized by projecting them on the markings of a stage micrometer or on the $\frac{1}{20}$ mm. divisions of an erythrocyte counting chamber. The ocular, objective, and tube length of the microscope must remain the same after this standardization. Measure the diameter of 500 or 1000 erythrocytes on a thin and evenly distributed blood smear.

Diffraction Method

The diffraction method determines the average cell diameter on a given field of a blood smear but does not give the maximum and minimum diameter of individual cells. The instrument used is termed an eriometer, halometer, or erythrocytometer (Fig 98). When a beam of light is passed through a blood smear, the diffraction of light produces concentric circles of rainbow colors, the diameters of which are inversely proportional to the average diameters of the erythrocytes on the field through which the light passes when the light source remains at a constant distance. By varying the distance of the light from the blood smear, one can make the size of the red circle coincide with a pattern of dots punched in a metal disk, and the instrument is calibrated to read the average red cell diameter in microns.

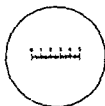


FIG. 97. Eyepiece micrometer scale for use in measuring the diameter of erythrocytes (E. H. Sargent and Co.)

BLEEDING TIME

Duke's Method

The most common way to determine bleeding time is by Duke's method. A stab wound, as for drawing blood for a cell count, is made in the lobe of the ear. At half minute intervals the blood at the site of the incision is blotted on a piece of filter or other absorbent paper. It is important not to make the slightest pressure near the incision or touch the skin with the absorbent paper. The bleeding time is the interval between the time of making the stab wound and the first time that the absorbent paper is not stained with blood when touched to the wound. The usual bleeding time by this

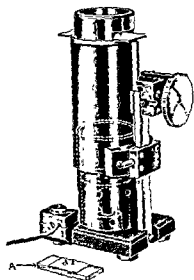


FIG. 98. Haden-Hausser erythrocytometer (A. H. Thomas Co.)

and is expressed as the percentage of the total blood volume or as the volume of erythrocytes in 100 cc. of blood.

Small tubes, such as the Wintrobe tube, may be used (Fig. 95). This requires only a small amount of blood and the use of heparin as an anticoagulant.

The van Allen hematocrit tube (Fig. 96) has proved very satisfactory in our experience. Blood from a finger or ear puncture is drawn into the tube to the top of the scale and is diluted with a small but unmeasured amount of 1.3% sodium oxalate solution. A spring sealing clip with a rubber cushion to prevent leakage is placed over the tip and the tube is placed in the centrifuge.

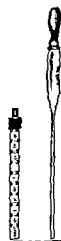


FIG. 95. Wintrobe hematocrit tube and filling pipet. The same tube is used for determining the sedimentation rate. (A. H. Thomas Co.)

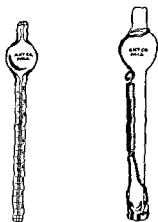


FIG. 96. Van Allen hematocrit tube and spring clip to prevent leakage. (A. H. Thomas Co.)

The upper level of the mass of red cells in the tube is read on the engraved scale on the stem of the pipet after centrifugation. This scale indicates directly the percentage of blood volume which is made up of packed erythrocytes. The normal volume of packed red cells per 100 cc. of blood is about 45 cc. for males and 41 cc. for females.

DETERMINATION OF RED CELL DIAMETER

Direct Measurement by Micrometer Eyepiece

An eyepiece micrometer disk, on which a scale is engraved (Fig. 97), is placed in the eyepiece of a microscope. The divisions on the scale are projected

drop of blood that appears. Touch the end of one of the capillary tubes to the next drop of blood. Blood will be drawn into the tube by capillary attraction. Carefully break off about half an inch of the tube after two minutes and continue breaking off a piece at half minute intervals until a fine thread of fibrin appears between the broken ends (Fig. 100). The coagulation time is the interval from the appearance on the skin surface of the drop of blood used for the test until a bridge of fibrin remains between the broken ends of the capillary tube. The glass tubing must be broken with care so as not to break the thread of fibrin. The normal coagulation time by this method is from three to five minutes.

Venous Blood Method, Lee and White's Method

Draw blood from a vein with a syringe and needle which have been rinsed in normal saline. Place 1 cc. of blood in each of two small Wassermann tubes



FIG. 100. Determination of coagulation time. Showing the strand of fibrin between the broken ends of the capillary tube indicating that coagulation has occurred.

of 8 mm. inside diameter which have also been rinsed with normal saline just before use. After the tubes have stood at room temperature for three minutes, tip one tube endwise every thirty seconds until the blood no longer flows and the tube can be inverted. The second tube serves as a check on the first. The coagulation time is the interval between the appearance of blood in the syringe and the time that the Wassermann tube can be inverted. The coagulation time of venous blood is longer than that of capillary blood since there is no tissue juice mixed with it. The normal is from five to eight minutes by this method. The clotting time is longer with tubes of greater diameter.

The coagulation time is lengthened in certain hemorrhagic diseases, notably hemophilia and prothrombin deficiencies. It is normal in thrombopenic purpura even though the bleeding time is markedly prolonged. There is no correlation between the bleeding and coagulation times.

CLOT REFRACTILITY

Place a few cubic centimeters of blood in a scrupulously clean Wassermann tube or small test tube. Allow this to stand undisturbed at room temperature and observe at hourly intervals. Under normal conditions the coagulum begins

method is from one to three minutes (Fig. 99). It may be prolonged to an hour or more in certain pathologic conditions, notably in thrombopenic purpura.

Ivy's Method, Venous Pressure Method

The cuff of a sphygmomanometer is placed around the arm as in taking a blood pressure reading, and a pressure which is just below the diastolic blood pressure is maintained so as to shut off the venous return. The skin of the flexor surface of the forearm a little below the elbow is cleansed with alcohol and wiped dry. A small stab wound, as in the preceding test, is made in the skin in the cleansed area and the blood blotted up with absorbent paper at half minute intervals. The bleeding time by this method is slightly

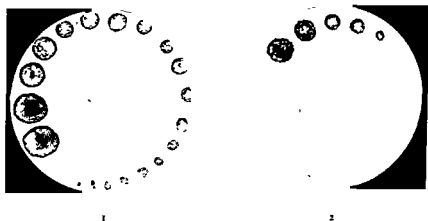


FIG. 99 Bleeding time by Duke's method 1. Prolonged bleeding time of $9\frac{1}{2}$ minutes
2. Normal bleeding time of $2\frac{1}{2}$ minutes.

longer than with Duke's method, the upper limit of normal is four minutes. Ivy's method is more accurate and will frequently show a prolonged bleeding time when there is a vitamin K deficiency even though the bleeding time by Duke's method is normal

COAGULATION TIME

Capillary Tube Method

Heat the center of a piece of glass tubing until it is soft and pull the ends apart quickly so that a long, fine capillary tube is formed having a diameter of about 0.5 to 1 mm. Break this into lengths of about 15 to 25 cm. Make a puncture wound in the finger or lobe of the ear and wipe away the first

mally begins at 0.44 or 0.42% saline and is complete at 0.34%. The concentration should be carried above the 0.5% level if hemolysis is present in this concentration.

A control series of tubes should be set up and a parallel test run on normal blood at the same time the test is being performed on the unknown. In this way a check on the accuracy of the saline solution may be obtained.

Erythrocytes are not hemolyzed in normal saline solution, but fluid is taken into the cells from hypotonic solutions until they rupture or hemolyze. A series of tubes containing varying degrees of hypotonic saline solution is therefore set up and the resistance of the cells to the varying concentrations of salt solution is determined. Cells which are more fragile than normal, as in hemolytic icterus, begin to hemolyze in 0.5% saline or even higher concentrations which more nearly approximate normal saline. In a few conditions, such as chronic anemia and obstructive jaundice, the cells may be more resistant than normal.

PLATELET COUNT AND VOLUMETRIC DETERMINATION OF PLATELETS

A platelet count is subject to such inaccuracies that it is difficult to get results that check closely. Because of this there are numerous methods and diluting fluids proposed for the procedure. There is a great tendency for the platelets to adhere to glass surfaces and to clump together. Consequently, an even distribution for counting is difficult to obtain.

Estimation from a Blood Smear

On the ordinary Wright-stained blood smear observe the number of platelets encountered while doing a differential count. They may be found singly, in small groups, or in large clumps and may be especially numerous at the point where the blood first came in contact with the glass slide. It should become a habit to observe the platelets on all blood smears and to estimate whether or not they are present in normal numbers. Because of the unequal distribution it is impractical to count the number of platelets in comparison to the number of erythrocytes.

Platelet Count—Olef Method

Prepare a small paraffin cup by melting out the center of a cube of paraffin with a hot glass rod. Place 2 or 3 drops of sodium metaphosphate solution in the cup. The solution is made as follows:

to retract and pull away from the sides of the tube at the end of one hour. At the end of four hours the clot has retracted from the walls of the tube and is almost completely surrounded by serum. The degree of retractility should be reported as absent, poor, or complete at the end of the four hour period. Observations beyond this period give little additional information. A clot which shows no retractility at the end of four hours is abnormal (Fig. 101) and is usually associated with a deficiency in the blood platelets.

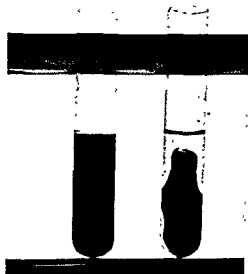


FIG. 101. Clot retractility. Showing a nonretractile clot (left) as compared to one with normal retractility (right).

After rinsed, add to the tubes the number of drops of distilled water necessary to make the volume of each tube 25 drops and shake each tube to mix the contents thoroughly. The percentage of salt solution in each tube is ascertained by multiplying its number by 0.02.

Obtain venous blood by venipuncture with a syringe and needle and place one drop of blood in each tube and mix. Let the tubes stand at room temperature for two hours. The nonhemolyzed erythrocytes will have settled to the bottom of the tubes at the end of this time, and any hemolysis will be evident by a reddish discoloration of the supernatant fluid. A faint pink color with a large collection of erythrocytes in the bottom of the tube indicates slight hemolysis. A red fluid with no sediment indicates complete hemolysis.

Record the strength of the solution in which hemolysis is first apparent and the first solution in which complete hemolysis is present. Hemolysis nor-

FRAGILITY TEST

Arrange a series of twelve small test tubes in a rack and number them from fourteen to twenty-five inclusive. With a capillary pipet place in each tube the number of drops of 0.5% saline solution that is indicated by the number on the tube. The salt solution must be accurately prepared and the size of the drops uniform. With the same pipet, after it is thoroughly

rinsed, add to the tubes the number of drops of distilled water necessary to make the volume of each tube 25 drops and shake each tube to mix the contents thoroughly. The percentage of salt solution in each tube is ascertained by multiplying its number by 0.02.

Obtain venous blood by venipuncture with a syringe and needle and place one drop of blood in each tube and mix. Let the tubes stand at room temperature for two hours. The nonhemolyzed erythrocytes will have settled to the bottom of the tubes at the end of this time, and any hemolysis will be evident by a reddish discoloration of the supernatant fluid. A faint pink color with a large collection of erythrocytes in the bottom of the tube indicates slight hemolysis. A red fluid with no sediment indicates complete hemolysis.

Record the strength of the solution in which hemolysis is first apparent and the first solution in which complete hemolysis is present. Hemolysis nor-

The depth of the fluid is 0.1 mm. and the dilution is 1:200.

Platelet count per cu. mm. = Platelets counted $\times 10 \times 200$.

Thrombocytocrit Method of Van Allen

The thrombocytocrit method is used routinely in this clinic in preference to actual counting of the platelets. Although minor variations in the number of platelets cannot be detected, the results are reliable and are satisfactory for clinical work. The method requires a special spherical sedimentation chamber of 20 cc. capacity and a special thrombocytocrit tube with a spring clip (Fig. 102).

Mix exactly 16 cc. of 1.3% sodium oxalate solution and 4 cc. of blood in the sedimentation chamber and allow this mixture to stand for $3\frac{1}{2}$ hours.

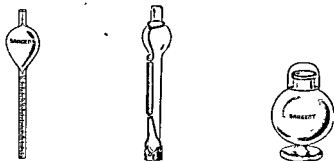


FIG 102 Van Allen thrombocytocrit tube, spring clip, and sedimentation chamber (E. H. Sargent and Co.)

During this time the erythrocytes settle out but the platelets are still equally distributed between the supernatant fluid and the erythrocytes. Place 5 cc. of the supernatant fluid in the thrombocytocrit tube, including just a trace of the corpuscular sediment so as to give a slightly reddish tinge to the fluid. Centrifuge at 3000 r.p.m. for $1\frac{1}{2}$ hours. The erythrocytes will be packed in the bottom of the tube and the platelets will appear as an ivory white column above them. The volume of the platelets is read directly on the scale of the tube in thousandths of a cubic centimeter, and the percentage of platelets per volume of whole blood is found by shifting the decimal point two places to the right. The normal range is from 0.4 to 0.6 per cent of the total blood volume.

HOWELL'S PROTHROMBIN TIME

Howell's prothrombin test determines the coagulation time of plasma to which an excess of calcium has been added. It is not a true determination of "prothrombin time" nor is it a measure of the prothrombin content of the blood.

Sodium metaphosphate	2.0
Sodium chloride	0.9
Distilled water	100.0

Let a drop of blood from the finger or ear drop into the solution, stir with a paraffin-coated wooden applicator. Transfer a drop of the mixture to a clean glass slide, cover with a cover slip, and seal with petroleum. Cut a square opening in a disk of paper or cardboard and place this in the ocular of the microscope so that the field of vision is reduced as in doing a reticulocyte count. With the oil immersion lens count the number of platelets and the number of erythrocytes in fields taken at random until 500 erythrocytes have been counted. Determine the number of erythrocytes per cubic millimeter of blood in the ordinary way. From the erythrocyte count and the ratio of platelets to erythrocytes on the moist preparation the number of platelets per cubic millimeter of blood can be determined.

Platelet Count—Direct Method

Method 1. Diluting fluid:

Aqueous solution brilliant cresyl blue (1:300) 2 parts

Aqueous solution potassium cyanide (1:1400) 3 parts

Keep the solutions in separate bottles, mix and filter just before using. The cyanide solution should be fresh every ten days.

Using an erythrocyte pipet draw the diluting fluid to the mark 1, then draw blood from a freely flowing puncture to the mark 0.5, and finally draw diluting fluid to the mark 101. Shake gently for two minutes, fill the counting chamber of the hemacytometer, and allow it to settle for ten minutes. Count with the high dry objective as in doing an erythrocyte count. Since the dilution is the same as for a red cell count, the calculations will be the same. The red cells are decolorized, the leukocytes are stained, and the platelets appear as rounded lilac-colored bodies. The normal count ranges from 150,000 to 250,000 per cubic millimeters of blood.

Method 2 Draw blood to the 0.5 mark in a red cell pipet and 2% sodium citrate to the mark 101. Shake slowly for two minutes. The shaking must be less vigorous than with an erythrocyte count as the platelets are more fragile and are easily destroyed. Put the suspension on a counting chamber and allow it to stand on a level surface for five minutes. The platelets appear as round sharply outlined refractile discs under the high dry objective. Count the platelets in the entire center square (the area used for erythrocyte counts) which is 1 square mm.

Wintrobe Method

Place 5 cc. of blood in a tube or bottle containing 6 mg. of ammonium oxalate and 4 mg. of potassium oxalate as an anticoagulant. Fill a Wintrobe hematocrit tube to the 10 cm. mark by means of a capillary pipet (see Fig. 95). Place the tube in a vertical position at room temperature and observe the point on the scale to which the corpuscles settle during one hour. After taking the reading, centrifuge the tube of blood until packing of the erythrocytes is complete and read the volume of packed erythrocytes (hematocrit). The sedimentation rate is corrected for the hematocrit reading by means of a chart (see original article).

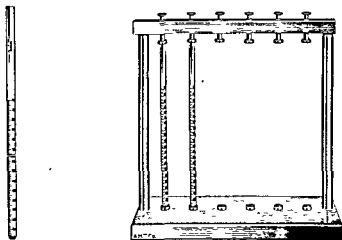


FIG. 103 Westergren sedimentation tube and rack (A. H. Thomas Co.)

Interpretation

Interpretation of the results of this test is somewhat difficult since there are many variables to take into consideration, the hematocrit, erythrocyte count, and hemoglobin content of the cells all influencing the sedimentation rate. The rate is more rapid in women than in men and is increased during menstruation and during the second and third trimesters of pregnancy. It is somewhat more rapid in children than in adults. There is an increased sedimentation rate with all types of infections, malignant neoplasms, wounds, operations, hemorrhage, coronary occlusion, and in anemia and leukemia. The test is of little value as an aid in differential diagnosis but finds its greatest usefulness in determining the degree of activity of various infections such

Collect 5 cc. of blood, place it in a centrifuge tube containing $\frac{1}{2}$ cc. of a 1% ammonium oxalate solution, and centrifuge. In four clean test tubes place 2, 3, 4, and 5 drops, respectively, of a 0.5% calcium chloride solution. To each tube add 5 drops of the blood plasma obtained by centrifugation. Note the time at which coagulation occurs in each of the four tubes. The prothrombin time is the elapsed interval between the adding of the plasma to the calcium chloride solution and the time coagulation occurs as determined by invertibility of the tubes. The normal time is less than twelve minutes but it is greatly prolonged in hemophilia.

Oxalated blood does not coagulate because the calcium has combined with the oxalate, but in this test an excess of calcium is added. It determines the time required for the platelets to break up and liberate prothrombin plus the time necessary for prothrombin to change to thrombin in the presence of calcium.

SEDIMENTATION RATE

Many methods have been devised to ascertain the sedimentation rate of the erythrocytes. The results are reported in various ways so that the method which is employed must be stated or the results reported in comparison to the normal values by the same method. When a column of blood is used, the results may record the distance the erythrocytes have settled in a given period of time, the time required for the erythrocytes to settle to an arbitrarily fixed point, or the height of the corpuscular column after a given period of time. Multiple readings may be taken at fixed intervals and a curve plotted from these data.

Westergren Method (Osgood Modification)

Westergren sedimentation tubes and a rack to hold them in a vertical position are necessary (Fig. 103). Obtain venous blood with syringe and needle and oxalate it (2 mg. of potassium oxalate per cubic centimeter of blood) to prevent coagulation. Draw blood to the zero mark in the Westergren tube, and place this in a vertical position in the special rack, which is equipped with a rubber cushion to prevent leakage. Read the upper level of the column of blood and record the time when the tube is put in place. Read the upper level of the red cells after fifteen and forty-five minutes. In a normal individual the erythrocytes will not settle over 5 mm. in a fifteen minute interval or over 15 mm. in a forty-five minute period. A sedimentation of over 30 mm. in forty-five minutes is abnormal.

STERNAL PUNCTURE

With the patient lying on his back prepare the area overlying the manubrio-sternal junction with antiseptic solution and drape with sterile towels. Infiltrate the skin, subcutaneous tissue, and periosteum with a local anesthetic. When anesthesia is complete, introduce a large bore needle, lumbar puncture needle shortened to 3.5 cm. or one of the specially made sternal puncture



FIG. 104 Constrictor test Showing petechiae which have appeared on the arm below the level of constriction.

needles, in the midline of the manubrio-sternal joint. Tilt the needle to an angle of 30 to 60 degrees and puncture the marrow cavity. Withdraw the stylet, attach a syringe to the needle, and aspirate 1 or 2 cc. of the marrow contents. Put the aspirated material into a small tube with 4 mg. of dry potassium oxalate or make smears directly from drops of the material from the needle. Cover the puncture wound with cotton and collodion after withdrawal of the needle. Hemoglobin determination, erythrocyte and leukocyte counts, and smears for differential counts may be made from the aspirated material which has been oxalated.

The aspiration may be done by inserting the needle directly through the

as in tuberculosis, pelvic inflammations, rheumatic fever, and infectious arthritis. It is of value in differentiating infectious or atrophic arthritis from hypertrophic osteoarthritis since the sedimentation rate is increased in the infectious type but not in the hypertrophic form.

BLOOD VOLUME

There are several methods for determination of the total blood volume. The dye method is the one most commonly employed and consists of injecting a known amount of dye and measuring its concentration in the blood plasma after a given period of time, usually after twenty minutes. Evans blue dye seems to be the most satisfactory as it is eliminated slowly. The concentration of the dye in the plasma is determined by a spectrophotometer. The plasma volume is ascertained from this reading and with the hematocrit reading on whole blood the total blood volume can be calculated.

The volume of the erythrocytes may be determined by the administration of a known amount of carbon monoxide gas and then measuring the amount of carbon monoxide per unit of blood.

A newer method uses radioactive iron or phosphorus to tag erythrocytes. Blood containing a known amount of such erythrocytes is transfused into the subject and the dilution of the tagged erythrocytes determined.

CAPILLARY RESISTANCE TEST

Constrictor Test, Arm Band Test, Rumpel-Leede Test

The cuff of a sphygmomanometer is placed about the patient's arm, as for taking a blood pressure reading, and inflated to a point slightly above the diastolic level. This pressure is maintained for three minutes. A rubber tourniquet may be used, but the pressure exerted must be below the systolic pressure. After removal of the cuff or tourniquet it is noted whether or not petechiae appear below the level of the constriction. Normally there will be none. The appearance of petechiae indicates an increased permeability of the capillaries (Fig. 104). A positive result may be expressed as the number of petechiae per unit area of skin, or the result may be graded from 1 plus (very few) to 4 plus (confluent or almost confluent areas of subcutaneous hemorrhage). A positive result will be obtained when there is a deficiency of platelets as well as with nonthrombopenic purpura. The test may be positive in scurvy, subacute bacterial endocarditis, severe anemias, and occasionally in normal individuals, particularly in women during hot weather.

Solutions

1. Stock solution: Dissolve 1 Gm of potassium bichromate in 50 cc of distilled water. Add 2 drops of concentrated H_2SO_4 and dilute to exactly 100 cc. with distilled water. This solution is stable when kept in a brown bottle.

2. Dilute standard: Dilute 1 cc. of the stock solution to 100 cc. with distilled water.

Obtain venous blood with a syringe and needle and either centrifuge or allow it to coagulate. Compare the color of the serum or plasma to that of the dilute standard in a colorimeter with the standard tube set at 15. If the color of the serum is too intense to read in the colorimeter it may be diluted.

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times \text{dilution} = \text{Icterus index}$$

Normal	2.5 to 5
Subclinical icterus	5 to 10
Visible icterus	10 and above

VAN DEN BERGH REACTION

The van den Bergh reaction differentiates between various forms of bilirubin. Water-soluble bilirubin gives a direct reaction and is present in the blood with obstructive jaundice. An indirect reaction is obtained with bilirubin before it has been acted upon by the liver. This type of reaction results from hemolysis of erythrocytes and from liver disease unassociated with biliary obstruction.

Procedure

Qualitative

Dialzo Reagent.

Solution A		Solution B	
Sulfanilic acid	0.5 Gm.	Sodium nitrite	0.5 Gm
HCl, concentrated	500 cc.	Water to 100 cc.	
Water to 1 liter			

Mix 3.3 cc. of solution A and 1 cc. of solution B immediately before use

In a centrifuge tube place 2 cc. of blood serum and layer with 1 cc. of diazo reagent. A positive direct reaction is shown by the appearance of a red or purple ring.

outer table of the sternum by a few sharp taps with the knuckles or with a small mallet rather than going through the manubrio-sternal joint. The special needles for this procedure have guards which prevent too deep insertion of the needle.

Pain is produced by the suction of the syringe in withdrawing the material. About 0.25 cc. of material is usually obtained.

Rubinstein has shown that aspiration from the crest of the sternum is equally as satisfactory as sternal puncture.

The results of differential counts on normal marrow as given by Osgood are found in the table below. A great variation in serial counts on the same material may be expected even though several hundred cells are counted and, as might be expected, the values given by other observers vary considerably.

	Percentage	
	Range	Average
Neutrophils—segmented	7-25.2	13.3
Eosinophils	0- 1.0	0.45
Basophils	0- 0.2	0.10
Neutrophils—staff (band) cells	15-35.0	24.10
Eosinophils " " "	0- 2.6	0.80
Basophils " " "	0- 1.0	0.06
Metamyelocytes—neutrophilic	1-10.0	7.40
Metamyelocytes—eosinophilic	0- 2.0	0.64
Myelocytes, neutrophilic	0-10.0	0.86
Promyelocytes, type I	0- 5.0	1.68
Promyelocytes, type II	0- 5.0	1.48
Myeloblasts	0- 2.0	0.44
Lymphocytes	4-16.0	10.60
Monocytes	0- 5.0	2.06
Normoblasts	5-20.0	12.40
Megaloblasts	0- 5.0	1.70
Disintegrating cells	10-30.0	20.80
Reticulocytes	1- 5.0	1.95
Myeloid-erythroblast ratio	2:1 9:1	3.6:1

Bone Marrow Biopsy

Biopsy of bone marrow is a surgical procedure in which a piece of red marrow is removed by a trephine opening into the sternum. The material removed may be fixed, stained, and studied histologically.

ICTERUS INDEX

The icterus index measures the intensity of the yellow pigmentation of the serum as compared to a standard solution of potassium bichromate.

DONATH-LANDSTEINER TEST

In paroxysmal hemoglobinuria the patient's blood contains an isohemolysin which unites with erythrocytes only at low temperatures and causes hemolysis of the cells after being warmed to 37° C. To detect the presence of the isohemolysin the Donath-Landsteiner test is employed.

Prepare a suspension of the patient's red cells in normal saline and also obtain several cubic centimeters of the patient's serum. Mix equal parts of serum and cell suspension and divide this mixture into two equal parts in test tubes. Keep one tube at room temperature as a control. Chill the other tube to 2° C (ice water or refrigerator) for ten minutes. Compare the color of the serum-cell suspension in the tubes, there should be no difference at this stage of the test. Place both tubes in a water bath at 37° C. A positive reaction is indicated by hemolysis occurring within thirty minutes in the suspension which has previously been chilled. There should be no hemolysis in the control tube. If no hemolysis occurs, add one-quarter volume of normal serum to each tube (to supply adequate complement), keep at 37° C for another thirty minutes, and note whether or not hemolysis has occurred in the tube which was previously chilled.

COLD AGGLUTININS

Prepare serial dilutions of plasma or serum in saline, beginning with 1:2 or 1:5 dilutions, placing 0.5 cc. of the dilution in 100 x 11 mm. test tubes. Add to each tube an equal amount of a 2 per cent cell suspension in saline using Group O cells. Shake the tubes and store in the refrigerator at 0° to 5° C. for at least eight hours. Place the racks of tubes in an ice water bath prior to reading. Invert the tube several times to loosen all of the cells from the bottom. The agglutination is graded from 1+ to 4+, the latter representing a single hard clump not broken up by mixing while the 1+ reaction is a floccular agglutination visible without magnification. The titer is the highest final agglutination giving a 1+ reaction.

After readings are made the tubes may be placed in a water bath or incubator at 37° C for two hours and the agglutination disappears.

PROTHROMBIN TEST FOR VITAMIN K DEFICIENCY

A deficiency of vitamin K leads to hemorrhagic tendencies through its effect on the prothrombin content of the blood. Simple tests for this condition

Mix the solutions and set aside until the maximum color has developed. Add 2 cc. of saturated ammonium sulfate solution, mix, and add 8 cc. of 95% alcohol. The appearance of the red color only after the addition of alcohol signifies an indirect reaction.

Quantitative

Mix the solution after the addition of alcohol, centrifuge, and to the supernatant fluid add 2 cc. of concentrated HCl and let stand for several minutes.

Standard Solution. Dissolve 10 mg. of pure bilirubin in 10 cc. of distilled water with 5 drops of 10% NaOH. Dilute to 100 cc. with distilled water. Add 50 cc. of freshly prepared diazo reagent, 100 cc. of saturated ammonium sulfate, and 400 cc. of alcohol. When a red color has developed, add 100 cc. of concentrated HCl. Let stand several hours before using. Store in ice box.

Compare the unknown with the standard solution in a colorimeter with the standard set at 20 mm.

Calculation:

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} \times 10 = \text{mg. of bilirubin per 100 cc. of serum or plasma}$$

Normal 0.5 mg. or less, indirect

HEMOSIDERIN IN URINE

Mix equal parts of 2% potassium ferrocyanide and 1% hydrochloric acid. Add to this the sediment from fresh urine and allow it to stand for fifteen minutes. Centrifuge and examine the sediment microscopically for the blue granules of hemosiderin.

SCHLESINGER'S TEST FOR UROBILIN IN THE URINE

To 2 cc. of urine in a test tube add 3 or 4 drops of Lugol's solution. To this mixture add an equal amount of a saturated alcoholic solution of zinc acetate. Centrifuge to throw down any precipitate or filter the solution. Throw a strong beam of light through the solution (the beam of light from an ophthalmoscope), a greenish fluorescence indicates urobilin. If bile pigments are present in the urine, they should be removed by adding one-fifth volume of 10% calcium chloride solution and filtering before performing the test.

elapsed time between the adding of the CaCl_2 solution and the appearance of coagulation is the prothrombin time. Repeat the test on the second tube and run a test on normal blood as a control.

$$\frac{\text{Prothrombin time of control}}{\text{Prothrombin time of patient}} \times 100 = \text{per cent of prothrombin activity}$$

Values of 85 to 100 per cent are normal. Values under 60 per cent are in the danger zone, and hemorrhage may occur. The normal clotting time is eleven to twelve seconds.

Link-Shapiro Modification of the Quick Method

This method of prothrombin determination is recommended by the American Heart Association for the control of dicoumarol administration in acute coronary occlusion. This differs in minor respects from the Quick method and the details may be found in the original articles listed in the bibliography.

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vised to determine the prothrombin time or prothrombin content of the blood.

Smith's Method

Thromboplastin for performing Smith's test is available from several manufacturers. Dissolve it completely in distilled water and add 0.1 cc. of the solution to each of two test tubes. To one tube add 0.9 cc. of normal blood immediately after it is drawn, mix, and note the time required for a clot to form. The normal time is twenty-five to forty seconds. To the other tube add 0.9 cc. of freshly drawn blood from the patient and observe the clotting time. The result may be expressed as the prothrombin time in seconds, but because of variations in the strength of the thromboplastin solution, it is best expressed by comparison with the normal.

$$\frac{\text{Clotting time of normal blood}}{\text{Clotting time of patient's blood}} \times 100 = \text{per cent of prothrombin activity}$$

Values below 70 per cent are considered to be an indication of vitamin K deficiency. Below 40 per cent is in the danger zone, and hemorrhage is apt to occur.

Quick's Prothrombin Method

Reagents

1. Calcium chloride solution. Dissolve 0.278 Gm. of CaCl_2 in 100 cc. of water.
2. Physiologic salt solution.
3. Sodium oxalate solution. Dissolve 1.34 Gm. of sodium oxalate in 100 cc. of water.
4. Thromboplastin. Add 0.15 Gm. of thromboplastin to 2.85 cc. of saline. Mix thoroughly, warm in water bath ($45-50^\circ \text{C}$), and let stand for sedimentation. Use the milky supernatant fluid for the test. This may be stored in a stoppered vial at 5°C .

Procedure

Obtain blood by venipuncture and to 0.2 cc. of sodium oxalate solution add 1.8 cc. of blood. Mix and centrifuge or allow the erythrocytes to settle. Into each of three test tubes $9 \times 65 \text{ mm}$ place 0.1 cc. of physiologic salt solution, 0.1 cc. of the patient's plasma, and 0.1 cc. of thromboplastin solution. Let stand for fifteen minutes, then warm in a water bath for one minute at 37°C . Warm the CaCl_2 solution to 37°C . and add 0.1 cc. to one test tube. Invert the tube at two second intervals until clot formation occurs. The

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